



The Nutritional, Physiological and Psychological Status of a Group of British Sappers after 23 Days of Adventure Training in the Hot Wet Tropics

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**CBRN Defence Centre
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ABSTRACT

The nutritional adequacy of both Australian combat ration packs and local feeding with fresh foods was evaluated during Exercise Pelopor Finn, a 23-day adventure training exercise conducted in Sabah, Malaysia. Thirty one males (aged 19 to 32 years) from the British 25 Engineer Regiment participated in the study. Blood samples were collected for determination of nutritional status and a skin immune-function test was performed before and after the exercise. Measurement of body mass, recording of food consumption, physical fitness testing, collection of saliva samples (immunoglobulin-A) and testing of psychological status (mood and cognition) occurred immediately before and after and at multiple time points during the study. Mean weight loss was 5.5%, decrements in physical and mental performance were not observed and good immune status was maintained. Food consumption was encouraged by the novelty of new foods, ability to socialise and take meal breaks, ability to self-select food items and number of serves, adequate sleep, good morale, and good hydration status. Conclusion: Although providing sufficient energy and macronutrients, the Australian CRP failed to provide sufficient iron, folic acid, antioxidants and vitamin B6 to prevent a decline in storage of these nutrients. Tobacco smoking and alcohol consumption were shown to be detrimental to nutritional status and alcohol may have had a particular negative effect on iron status.

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Executive Summary

Although troops engaged in land operations should expect to be fed to the best possible standard, using fresh foods wherever possible, there are situations when fresh rationing is not possible, or when its introduction will be delayed because the tactical situation does not allow field kitchens to be established for many weeks after deployment. Furthermore, the Army after Next requirement is for rapid force projection, increased mobility and improved sustainment. The re-supply of smaller and more highly dispersed fighting units places an emphasis on innovative feeding systems and greater use of combat ration packs (CRP).

The effect on military performance and long-term health of extended CRP feeding is not known. The present study was designed to investigate the effects of longer-term feeding with CRP. The study was conducted in association with Exercise Pelopor Finn (meaning "trail blazer", EX PF) an adventure training exercise conducted by a British engineer unit (25-Engineer Regiment) in Sabah, Malaysia. EX PF presented an opportunity to test the acceptability of the Australian combat ration one man (CR1M) under field conditions devoid of many of the usual operational stresses. Thirty-one male sappers participated in the study. Twenty sappers were at Tawau Hills Ranger Station (THRS) and were fed solely with CR1M and eleven were at the Danum Valley Field Centre (DVFC) and were fed with local fresh foods. The two locations were five hours apart by four-wheel-drive vehicle.

Close control was not maintained over rationing, and hence sappers were able to access more than one CR1M per day at THRS and self-selected serves of food from the kitchen at DVFC. A weekly barbecue meal was provided for sappers at DVFC, with beer and soft drinks. In addition, all sappers at DVFC were given brew kits containing tea, coffee, biscuits, chocolate, milk, sports drink and sugar.

The engineering tasks involved construction of an ecological trail (THRS), construction of observation towers 40 to 60 m above ground in rainforest trees (DVFC) and a survey of roads in the Danum Valley conservation area (DVFC).

The DVFC group did not constitute a control for evaluation of CR1M; however they did provide important information relating to food acceptability, consequent energy balance and nutritional status measures.

Blood samples were collected for determination of nutritional status (vitamins, cytokines, visceral proteins, and ferritin) and a delayed hypersensitivity skin test (immune function) was performed before and after the exercise. Measurement of body mass, recording of discarded ration items at THRS (packaging and foods) and recording of foods eaten at DVFC, physical fitness testing, collection of saliva samples (immunoglobulin-A) and testing of psychological status (mood and cognition) occurred immediately before and after and at multiple time points during the 23 day study. The measurement outcomes were body mass, nutrient intakes, immune function, micronutrient and visceral protein status, hydration status, aerobic capacity, mood states and cognitive ability.

At both locations sappers ate about 90% of their mean estimated energy requirement (between 0.8 and 1.3 ration packs per day at THRS). This ensured that weight losses were less than 10% of body weight over 23 days (average loss of 5.5%) and decrements in physical and mental performance were not observed. Furthermore good immune status was maintained throughout the exercise. Factors which may have encouraged food consumption included the novelty of eating new foods, ability to socialise and take meal breaks, ability to self-select food items and number of serves, adequate sleep, good morale, and good hydration status. However, tobacco smoking and alcohol consumption contributed to under consumption of food and alcohol consumption may have had a particular negative affect on iron status.

Some conclusions could be drawn about the adequacy of the CR1M:

- To meet the energy requirements of an engineering task involving prolonged moderate to hard physical activity, a group of British sappers needed to eat more than the contents of one CR1M ration per day.

- Under field conditions where soldiers consume one or more ration packs per day, the CR1M provides sufficient energy and macronutrients to prevent serious protein energy malnutrition for up to 23 days of moderate to high physical activity in a hot tropical environment but fails to provide sufficient iron, folic acid, antioxidants and vitamin B6 to prevent a decline in storage of these nutrients.

Further study is required to determine the implications of operational anorexia (ie decreased appetite), declining vitamin and mineral storage (particularly iron), weight loss and suppressed immune function on redeployment. To achieve this, the causes of operational anorexia need to be better understood and the effects of long-term feeding with CRP under operational conditions need to be better assessed.

Authors

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Christine graduated from the University of Queensland (UQ) with BSc(Hons) and PhD (1992) in biochemistry (enzymology - cofactors and vitamins). She has also obtained qualifications in education (Dip Ed, UQ) and dietetics (Grad Dip Nutr Diet, QUT). She has membership of the Australasian Association of Clinical Biochemists, American Association of Clinical Chemists, Dietitians' Association of Australia, Nutrition Society of Australia (Secretary Tas branch), the Australian Institute of Food Science and Technology, Tasmania's Food Advisory Council and is an Honorary Research Associate in the School of Human Life Sciences, University of Tasmania. Christine has held research positions within UQ and QUT and a supervising scientist position within Chemical Pathology at Royal Brisbane Hospital. In her four-years employment as senior chemist at the Defence Nutrition Research Centre Christine has been investigating the nutritional status of soldiers and the effects of long-term combat rationing on health and military performance.

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1. Introduction

The most recent White Paper [1] refers to “the Government’s intention to retain the ADF as a first-class military force, able to fight and win”. “In return, the Government demands that resources, including people, are managed efficiently to ensure that our uniformed personnel, especially in operational and supporting units, are properly trained, equipped and looked after”.

The White Paper identifies more effective recruiting, better training, and higher retention of skilled, experienced ADF members as integral human factors (HF) aspects that must be addressed for the ADF to remain ‘a force to win’. However, there is another, deeper level of HF that is fundamental to operational readiness—the health and fitness of the individual ADF member. Being skilled and experienced will be of limited value during operations if the member is not also physiologically and psychologically ‘fit to fight’.

In the context of operational readiness an ADF member has a high level of fitness if he/she:

- is healthy (i.e. there is no overt illness);
- possesses appropriate physical characteristics (including body dimensions and appropriate levels of body fat and lean body mass for the operation);
- is physically fit for the intended operation (e.g. having high aerobic fitness if endurance marching is involved);
- is psychologically fit (including concepts such as vigilance, cognitive performance);
- has optimal nutritional status; and
- has optimal immunological status.

Factors that have major influence over the above variables include preventive health measures (to avoid illness), physical fitness training, and the quantity and quality of the diet. It is this last point together with the effects of sub-optimal diet on performance during sustained operation that is addressed in this paper.

ADF members engaged in land operations may expect to be fed to the best possible standard, using fresh foods wherever possible [2]. However, there are situations when fresh rationing is not possible, or when its introduction will be delayed because the tactical situation does not allow field kitchens to be established for many weeks after deployment. As an example, at the start of Operation Warden, ADF members of

INTERFET were rationed with Combat Ration One Man (CR1M) for up to 35 days (*pers. comm.* ADF Catering Group, DMO) or even longer (self-reported in a survey of members of 2AFDS, 3RAR and 2RAR). The CR1M provides about 15 MJ per day with up to 60% of energy being derived from carbohydrate [3]. This is adequate energy and a suitable carbohydrate content for ADF members engaged in land operations that involve moderately-vigorous work (typical of most peace-time field exercises).

The periods of rationing with CR1M during Operation Warden are well in excess of those officially sanctioned. SUPMAN 4 [2], states that CR1M should be used for a maximum of 16 consecutive days during peace and 20 days during war. However, SUPMAN 4 also acknowledges that during war emergency, combat ration packs (CRP) may be used for rationing 'as necessary' (i.e. there is no time limit if no alternative form of rationing exists).

The effects on military fitness of long-term feeding with CRP have not been determined [4]. In response to international concern about this situation, Group HUM (abbreviation of Human Resources and Performance) of The Technical Cooperation Program (TTCP) formed Action Group 16 (AG16) to review the current state of knowledge in this area. This was an Australian initiative, and AG16 was chaired by Australia for the three years of its existence. The final report concluded that when rationing is by CRP "25% or more of the food provided is commonly discarded by end users ... As a result, under-consumption—leading to weight loss and, eventually, to decrements in physical and cognitive performance—appears to be almost inevitable when rationing is with CRP that have to be carried by troops. Exacerbating this is the observation that carbohydrate—the nutrient of greatest significance to both physical and cognitive performance—is often the nutrient at most risk of being discarded [4]."

Therefore, simply because a ration is nutritionally adequate (as assessed by nutritional analysis) does not necessarily mean that it will sustain an ADF member indefinitely. The consistent finding that 25% or more of the available food is discarded, with carbohydrate (the preferred fuel for muscular and cognitive work) as the nutrient at greatest risk, throws into doubt the adequacy of CRP to sustain ADF members on physically-arduous operations, even over the short-medium term.

Controlled randomised studies are essential to determine the effects on military fitness of rationing with CRP during sustained operations. In their final report, AG16 recommended that: "Research should be continued on the nutritional, physiological, psychological and immunological implications of feeding with CRP on sustained operations" [4].

Australia agreed to take the lead in investigating the effects on operational performance of long-term feeding with CRP.

In 1999, in collaboration with the US Army Institute of Environmental Medicine, a study was conducted on the effects of eating the Combat Ration One Man (CR1M) as

the sole source of food in the medium term [5]. This study was conducted at RAAF Base Scherger, in conjunction with Exercise Northern Awakening (EX NA). The study evaluated the effects of 12 consecutive days of eating CR1M as the sole source of nutrition on nutritional, biochemical, physiological psychological and immunological status of Airfield Defence Guards (ADGs). To control for non-nutritional factors, ADGs received either meals prepared from fresh foods ('Fresh' group, 15 MJ, n = 13), complete CR1M ('Full CRP', 15 MJ, n = 10) or half CR1M ('Half CRP', 7.5 MJ, n = 10).

The availability of 15 MJ was calculated by the Ration Expert Advisor Program (REAP™), a US expert system designed to predict food and water requirements based on input data such as duration of operation, method of locomotion, load carriage, terrain, climate and so on. One aim of the study was to determine how accurate REAP™ was in predicting nutrition requirements.

Mean daily energy consumption was highest (12 MJ) for the Fresh group. They evidently matched their energy intake with expenditure, as they did not lose weight. The Fresh group also maintained their nutritional and immunological status. A high rate of ration item discards by ADGs in the Full CRP group resulted in ADGs having a mean intake of 9.2 MJ/day (61% of the available energy). Reduced access to food in the Half CRP group led to a mean intake of only 6.6 MJ/day (88% of available energy).

The three groups had similar levels of physical activity, similarly disrupted sleep patterns and poor sleep quality. No changes were observed in cognition and there were no discernible differences in cognitive performance between dietary treatments.

Reduced food consumption by the ADGs in the two CRP groups resulted in mild symptoms of weight loss, suppressed immune function, loss of visceral protein, increased fatigue, loss of vigour, and increased feelings of confusion. Carbohydrate intake was negatively associated with run times during the fitness testing.

However, despite minor differences, there were no substantial differences among the treatment groups in their mental or physical performance. This implies that ADF members engaged in typical field exercises can cope for at least 12 days on approximately half rations, drawing on their nutritional reserves and/or adapting to the reduced food intake by using nutrients more efficiently.

Booth et al [5] concluded that a similar study was needed over a much longer period to determine when significant decrements to performance are likely to occur. They also recommended incorporating into future studies an evaluation of the potential of alternate CRP items or supplements to ameliorate, or reverse, adverse metabolic and psychological effects associated with impaired military performance.

The present study was designed to investigate the effects of long-term feeding with both freshly prepared meals and CRP on military performance. The opportunity was also taken to investigate the field acceptability and effects on physical and cognitive

performance of a high-carbohydrate supplementary ration pack item. This was a commercially-manufactured HooAh!TM bar, produced by US industry for the US Army Soldier Systems Command, Natick MA. Non-commercial HooAh!TM bars had previously been trialled at EX NA, with promising results [5].

The study was conducted in association with Exercise Pelopor Finn (meaning 'trail blazer', EX PF), an adventure training exercise involving British soldiers from an engineer unit (25-Engineer Regiment). EX PF was conducted in Sabah, Malaysia, in the period Feb-Mar 2000. Conducted in collaboration with the Defence Evaluation and Research Agency-Centre for Human Sciences (DERA-CHS), and the Malaysian Defence Science and Technology Centre (DSTC), the aims of this study included determining the:

- nutritional adequacy of the Australian CR1M as the sole source of nutrition over a 3-4 week period for a group of engineers engaged in trail construction in a hot/wet jungle environment;
- effectiveness of a high-energy carbohydrate supplement, the HooAh! bar, to increase the voluntary intake of carbohydrate by soldiers working in a jungle environment;
- effects of heat and the workload on hydration status of engineers and the water requirements for engineering work in a hot/wet jungle environment; and
- effectiveness of practical tools for evaluating hydration status in the field.

2. Methods and Materials

2.1 Participants and Study Design

Experimental procedures were approved by the Australian Defence Human Research Ethics Committee (ADHREC protocol 186/99). Copies of the information and consent forms are included in Appendix A.

Thirty-one male sappers (aged 19 to 32 years) were recruited from the British 25 Engineer Regiment (Regular Army), who had been selected to take part in Exercise Pelopor Finn. The sappers were located at two sites five hours apart by four-wheel-drive vehicle: Tawau Hills Ranger Station (THRS, $n = 20$, mean age 27 years) outside Tawau and the Danum Valley Field Centre (DVFC, $n = 11$, mean age 26 years) outside Lahad Datu. Sappers were rationed with CR1M at THRS and with freshly prepared meals at DVFC.

Close control was not maintained over rationing, and hence sappers were able to access more than one CR1M per day at THRS and self-selected serves of food at DVFC. Sappers at DVFC had access to a kitchen and food to prepare their own breakfasts. Lunches were generally provided in the field, and consisted of filled bread rolls or fried noodles on most days. Evening meals were prepared in the DVFC kitchen, and once each week a barbecue meal was provided, with beer and soft drinks also being available at the barbecue. Some members of the survey team spent up to five days at a time in the field and consumed CR1M on those days. In addition, all sappers at DVFC were given brew kits containing tea, coffee, biscuits, chocolate, milk, sports drink and sugar.

The engineering tasks involved construction of an ecological trail (THRS), construction of observation towers 40 to 60 m above ground in rainforest trees (DVFC) and a survey of roads in the Danum Valley conservation area (DVFC) [6].

The DVFC group did not constitute a control for evaluation of CR1M; however they did provide important information relating to food acceptability, consequent energy balance and nutritional status measures.

Two high carbohydrate supplements, HooAh!TM bar and Ergo DrinkTM (supplied by US Army Soldier Systems, Natick) were provided to participants at THRS. The original work plan for THRS had sappers divided into trail teams of four, and a cross-over design based on this plan was set up to test the effect on voluntary carbohydrate intake of the high-energy carbohydrate supplements. Within the first few days of work at THRS the work plan was abandoned to allow greater flexibility, resulting in the carbohydrate study also having to be abandoned. The two supplements were made freely available to sappers at THRS for the remainder of the study. These participants

completed a brief questionnaire concerning the acceptability of the two products on the final day of the study.

2.2 Dietary Analysis

Participants in the test group returned their food packaging and uneaten items within name-labelled bags. Initially the sappers were located close to the field laboratory and returns were possible every two to three days. By the completion of the study they were up to six hours walk over difficult terrain from the field laboratory. As a result, returns became less frequent. As far as the incomplete data set allowed, the amount of food consumed and discarded was recorded as daily means of macronutrients for each participant.

The dietary intake of the DVFC group was estimated from immediate recall data and sample pre-weighed meals. At the conclusion of each meal sappers were asked to state the amount of each food item consumed. Members of the survey team provided a written list of foods consumed during periods spent in the field.

Nutrient intake was calculated with Foodworks Version 2.10 (Xyris Software, Brisbane, Australia), using the Nuttab95, AusFoods, AusNut, ADFnut and Malaysian databases plus additional food recipes created in Foodworks from base ingredients.

2.3 Physical Fitness and Body Composition Testing

Tests of physical fitness (sit-ups, standing vertical jump and hand-grip – see subsection 2.3.3), body mass and body composition (by bio-electrical impedance, BIA – subsection 2.3.1) were conducted on the combined group (n = 31) on the second day after arrival in Sabah (pre-study, at Malaysian army barracks, Kota Kinabalu) and again on the final day of the study (day 23 at THRS). Height had already been measured in the UK. Fasting blood, saliva and urine samples were collected and delayed-type hypersensitivity skin tests were also conducted on these days.

At THRS, the multistage fitness test (subsection 2.3.2) was conducted along with vertical standing jump, situps and hand-grip on days 3 and 10. Body mass and body composition by BIA were also measured on these days.

At DVFC, the only fitness tests that could be conducted were vertical standing jump and sit-ups. These were conducted on days 3, 8, 10, 17 and 20, and body mass was recorded daily after the first week.

2.3.1 Body composition

Body mass (kg) was measured by use of calibrated scales (AND, model CH-150K, A&D Mercury Pty. Ltd., Australia). Sappers were requested to towel-dry and were weighed in underwear after voiding their bladders. Bioelectric impedance was measured by use

of the Seac BIM 4 bioelectric impedance analyser (BIA) (Seac Pty Ltd and Uniquet Ltd, Qld, Australia). The instrument uses a single electric frequency of 50 kHz and uses the Lukaski prediction equation to estimate percentage body fat from a calculated estimate of total body water [7]. A calibration check was performed immediately before each measurement session and [8].

Sappers were tested in a well-hydrated, post-prandial state. They were instructed to have a large drink of water at least an hour before measurement, with no further drink or food to be consumed within one hour prior to measurement. The participant lay face-up with legs slightly apart and hands resting next to the trunk, palms down. Care was taken to ensure that the hands were not touching any part of the body. The inner thighs were not permitted to be in 'skin-to-skin' contact. The participant had removed his right shoe and sock. Electrodes connected to the BIA were placed on the right hand and right foot as described in the manufacturer's instruction manual.

2.3.2 Multistage fitness test

This test, commonly referred to as the "shuttle run" or "beep test", was conducted to estimate sappers' aerobic capacity [9]. The test was conducted at THRS (the only location which had a suitable surface). Participants were required to run back and forth between two lines 20 m apart at progressively faster speed until volitional exhaustion. They were guided through this protocol by matching their shuttle runs with audible beeps. Each participant's final speed was used to estimate aerobic capacity by use of a regression equation [9].

2.3.3 Tests of upper-body muscular strength and endurance, and lower-body power

Abdominal muscle strength and endurance was tested by recording the maximum number of correctly performed sit-ups completed within two minutes, and single jump power was tested by recording the greatest of three standing vertical jumps. Hand-grip strength was determined using a hand-grip dynamometer (Jamar Hydraulic Hand Dynamometer, Canada). The sappers had two attempts with each hand to exert maximal force on the dynamometer. The higher result was recorded as 'hand-grip strength' for each hand.

2.4 Estimation of Strain, Environmental Thermal Stress and Hydration Status

Physiological strain and hydration status were determined on selected days by measurement of core body temperature (THRS only), heart rate monitoring, urine analysis and hydration calculations. Wet bulb globe temperature (WBGT) was determined as an indication of environmental thermal stress. Ratings of perceived exertion, thermal sensation and comfort were recorded by sappers at DVFC.

2.4.1 Core body temperature and heart rate monitoring

Gastro-intestinal temperature was measured using a system developed by HTI Inc., USA and PED Inc., USA which uses a pill that emits a low power radio signal, the frequency of which is temperature-dependent. The signals were captured continuously by a body core temperature monitor (BCTM, PED, USA). Sappers were instructed to swallow a pill on the evening prior to participation to ensure that the pill had left the stomach and had entered the intestine before the measurement period began. In this way, sudden changes to temperature as a result of eating or drinking were avoided.

Heart rate (HR) was recorded at one-minute intervals from the R-wave frequency of ventricular depolarisation (Polar Sport Tester ®). The transmitter was fastened around the sapper's torso and the receiver was attached to the wrist. HR data were downloaded to a laptop PC at the completion of each day's recording.

2.4.2 Urine analysis

Urine samples were collected at least every third day—with some individual exceptions—due to participant unavailability—and were transported cold in foam boxes to the field laboratories. Samples were analysed to determine hydration status by specific gravity and osmolality. The same samples, which had been stored and transported frozen, were later analysed in the DNRC laboratory for total nicotine metabolites and creatinine.

Specific gravity was measured by use of a hand-held refractometer (Uricon-NE Specific Gravity Urine Specific Gravity Refractometer, Atago Co. Ltd, Australia). Osmolality was measured using an Advanced Micro-Osmometer Model 3300 (Advanced Instruments Inc, MA, USA). An SG in the range 1.003 to 1.030 g/L and an osmolality of <1,000 mmol/kg were used as the criteria for good hydration status [10]. Total nicotine metabolites were determined using a colorimetric assay adapted for an automatic chemistry analyser (Cobas Bio, Roche, Australia) [11].

2.4.3 Hydration calculations

Sweat rate cannot be determined directly in the field. It can most conveniently be estimated by weight loss (disregarding the small loss in weight attributable to CO₂ production). Total sweat loss (L) over the period of observation was calculated as:

Initial nude weight - final nude weight + water intake + solid intake - urine output, where all variables were measured in kg. (Note that 1 L of urine or sweat ~1 kg).

2.4.4 Environmental thermal stress

The ambient temperatures at both locations were measured on selected days using a Metrosonics hs-3700 portable heat stress monitor (Metrosonics Inc., NY, USA). The wet

bulb, dry bulb and globe temperatures were recorded, from which the WBGT index of environmental stress was calculated. The environment is considered thermally stressful above a WBGT of 26 - 27 °C. Windspeed was recorded using a wind-vane anemometer (Met One Instruments, USA).

2.4.5 Rating of relative perceived exertion (RPE)

Borg's subjective RPE, which is a reliable indicator of exercise tolerance, was completed by sappers at DVFC during observation days [12]. Perceived exertion ratings correlate highly with exercise heart rates and work rates. The RPE scale was developed to allow the exerciser to subjectively rate his/her feelings during physical activity, taking into account personal fitness level, environmental conditions and general fatigue levels. Physical activity intensity is rated on a scale of 6 (with 7 being *very, very light*) to 20 (with 19 being *very, very hard*).

2.4.6 Rating of thermal comfort (TC)

The rating of TC, which is a 5-point subjective scale, rates how comfortable a person feels with the perceived temperature of their body on a scale of 1 (*comfortable*) to 5 (*extremely uncomfortable*) [13].

2.4.7 Rating of thermal sensation (TS)

The rating of TS allowed sappers to communicate how hot they felt on a scale of 1 (*unbearably cold*) to 12 (*unbearably hot*) [13].

2.5 Energy Expenditure

Total energy expenditure (TEE) was estimated on selected days using a factorial method [14]. The sappers' activities and the duration of those activities were observed and recorded on days designated as 'thermal strain' days. The observed activities were assigned energy expenditure values by matching them to the energy costs of activities reported in the literature.

2.6 Immune Function and Nutritional Status

Immune status was determined by measurement of cell-mediated immunity (delayed-type hypersensitivity skin test and plasma cytokines) and humoral immunity (salivary secretory immunoglobulin A, sIgA). Nutritional status was determined by measurement of the body's stores of visceral protein (plasma insulin-like growth factor 1, IGF-1, and fibronectin), iron (ferritin) and vitamins (riboflavin, vitamin B₆, folic acid, total antioxidants and vitamin K). Fasting blood samples were collected before the study (Kota Kinabalu Army barracks) and on the final day of the study (day 23 at THRS). Saliva samples were collected at both locations on days 1, 3, 4, 5, 6, 9, 13, 16, 19 and 21.

2.6.1 Delayed-type hypersensitivity skin test

When healthy persons are re-exposed to recall antigens administered intradermally, an immune response is stimulated. This is typically in the form of a delayed-type-hypersensitivity (DTH) response with an area of induration and erythema occurring after 48 hours. DTH skin-test reaction is an *in vivo* test for immune competence and function of T-lymphocytes and macrophages. The Multi-test Cell Mediated Immunity DTH Skin test kit (CSL Biosciences) consists of a disposable plastic applicator with eight sterile test heads. The heads are loaded with seven test antigens (tetanus toxoid, diphtheria toxoid, streptococcus, tuberculin old, candida, trichophyton and proteus antigens) and a glycerin negative control. The applicator is pressed firmly against the skin of the inner forearm and a small amount of soluble antigen is introduced by puncture. Circulating T-cells (lymphocytes) sensitised to the antigen from prior contact react with the antigens in the skin and induce a specific immune response, which manifests as a small red inflammation. The red area (2-10 mm diameter) is measured 48 hours after application.

2.6.2 Blood and saliva tests

Samples were centrifuged on-site and the separated RBCs, plasma and saliva were stored frozen (-20°C) then air-transported frozen to DNRC for analysis.

Total plasma homocysteine (Hcys) was defined as the sum of all homocysteine species in plasma, including homocysteine, homocystine, mixed disulfides, and protein-bound forms. All were converted to Hcys by reduction with sodium borohydride, then measurement by HPLC with fluorescence detection using a method adapted from Allena et al. [15]. Ferritin and fibronectin were measured by particle-enhanced nephelometric assay using manufacturer-supplied reagents (Behring BNA, Dade Behring, Germany). Human interleukin-2 (IL2) and interleukin-6 (IL6) were measured by competitive enzyme immunoassay (Accucyte kits, Cytimmune Sciences Inc, Maryland USA). Interleukin 2 receptor (IL2r) was measured by endpoint enzyme immunometric assay (Milenia kit, DPC, Los Angeles, USA). IGF-1 was measured by enzyme-linked immunosorbent assay using a non-extraction procedure (Diagnostic Systems Laboratories, Webster, Texas, USA). De-carboxy prothrombin or PIVKA-II (Protein Induced in Vitamin K Absence - factor II) was measured by enzyme-linked immunosorbent assay (Asserachrom PIVKA-II, Diagnostica Stago, France). Total antioxidant capacity (TAOC) was measured by incubation of plasma with 2,2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS®) in the presence of a peroxidase and hydrogen peroxide to produce the radical cation ABTS®⁺. This colorimetric assay used reagents supplied by Randox Laboratories, UK. Red blood cell riboflavin and vitamin B₆ were determined by enzyme activation assays using a Cobas Bio fast scan centrifugal analyser (Roche Diagnostics, New Jersey, USA).

Unstimulated saliva collection was into Salivette tubes (Starstedt, Germany). Sappers were asked to rinse their mouth, swallow until dry and then place a cotton swab in their mouth, leaving it in place without chewing until they needed to swallow. The participant then placed the cotton swab back into the tube. Albumin (Alb) and sIgA were measured by nephelometric assay using manufacturer-supplied reagents (antisera to human IgA α chain and human albumin, Dade Behring, Germany). The results were presented as the ratio of sIgA (mg/L) to Alb (mg/L).

2.7 Psychological Measurements

Three questionnaires were administered to all sappers at the same time on days 2, 4, 8, 12, 16 and 20: Army Speed and Accuracy (ASA) questionnaire, State of Fatigue Inventory (SOFI, Swedish Occupational Fatigue Inventory-20, Arbetslivsinstitutet 1998) and Profile of Mood States (POMS - short form, McNair, Lorr and Droppleman, Edits., CA USA). The Hopkins symptoms general health checklist was administered on days 2 and 20.

2.8 Statistical Analyses

Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences, version 9.0, 1999, SPSS, Inc., Chicago, IL). Descriptive statistics were obtained to establish a measure of central tendency and are presented as means, standard deviations, standard error and range. Data were checked for outliers and non-homogeneity of the population by use of pair wise scatter plots, box plots and Q-Q plots. Normality of population distributions was checked by the Shapiro-Wilk and Lilliefors tests. Where appropriate data were log transformed to achieve normality.

Significance was accepted at $p < 0.05$. Multiple linear regression analyses were used to assess associations between variables. Comparison of means was achieved by use of the paired t test and Levene's test was used for comparison of variance. In order to determine the statistical difference between groups at the two locations, univariate analysis (general linear model) with LSD post-test was applied to the change in response in the case of variables recorded at baseline and completion of the study. Repeated measures analysis of variance was used to compare dietary treatments for tests with serial measurements.

3. Results

3.1 Dietary Intake, Energy Balance and Body Composition

Mean daily dietary intakes remained stable for sappers at both THRS and DVFC throughout the study, with the exception of dietary fat intake, which declined at DVFC towards the end of the study (Figs 1 and 2). Apart from fat ($F = 16.169$, $p = 0.021$) and alcohol (available only at DVFC) there were no differences in consumption of macronutrients between the two sites. Table 1 summarises the mean daily intake of macronutrients at the two field locations.

Sappers at both locations appeared to adjust their dietary intake to meet their requirements and as a result body mass losses were small. The group mean change in body mass at THRS was -5.4% ($SD = 2.4$, range 0.3% to 9.6% , $t = -8.419$, $p < 0.001$) and at DVFC it was -4.3% ($SD = 2.1\%$, range 0.6% to 7.6% , $t = -6.212$, $p < 0.001$). Measured losses in body fat were not significant and this was most likely due to the poor sensitivity of the BIA method.

Sappers who smoked tended to eat less carbohydrate than others. Concentration of total nicotine metabolites in urine was negatively correlated with carbohydrate consumption and energy consumption (Fig 3, $r = -0.692$ and $r = -0.697$ respectively, both with $p < 0.001$).

Table 1 Daily macronutrient intake by sappers at Tawau Hills Ranger Station and Danum Valley Field Centre

	Tawau Hills Ranger Station				Danum Valley Field Centre				
	Energy (MJ)	Protein (g)	Fat (g)	Carbohydrate (g)	Energy (MJ)	Protein (g)	Fat (g)	Carbohydrate (g)	Alcohol (g)
N	20	20	20	20	11	11	11	11	11
Mean	15.7	114	136	564	13.9	119	114	427	52.4
SD	3.5	37	37	133	4.5	47	47	144	36.4
SEM	0.8	8	8	30	1.4	14	14	44	11.0
Median	15.9	115	136	573	13.1	112	105	427	43.0

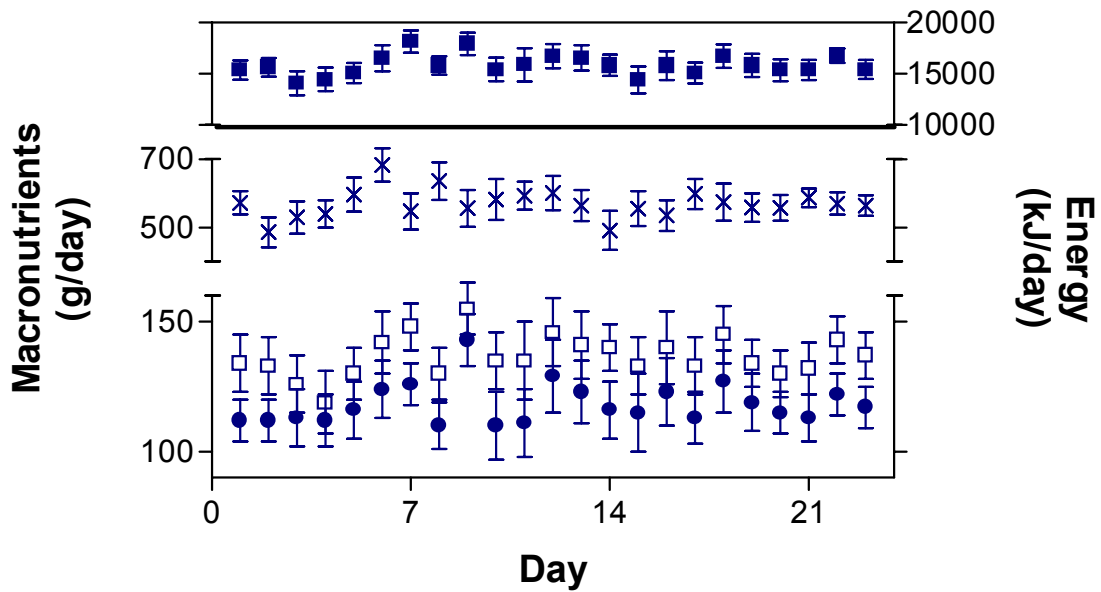


Figure 1: Mean and SEM for daily macronutrient intake by the sappers at Tawau Hills Ranger Station. Energy ■, Carbohydrate *, Fat □, Protein ●

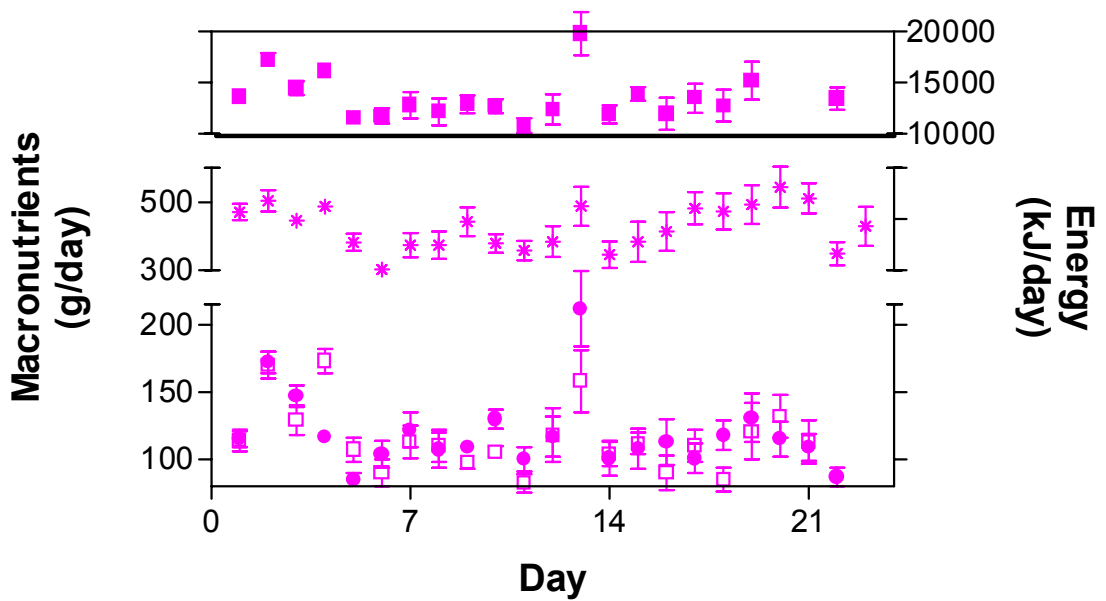


Figure 2: Mean and SEM for daily macronutrient intake by the sappers at Danum Valley Field Centre. Energy ■, Carbohydrate *, Fat □, Protein ●

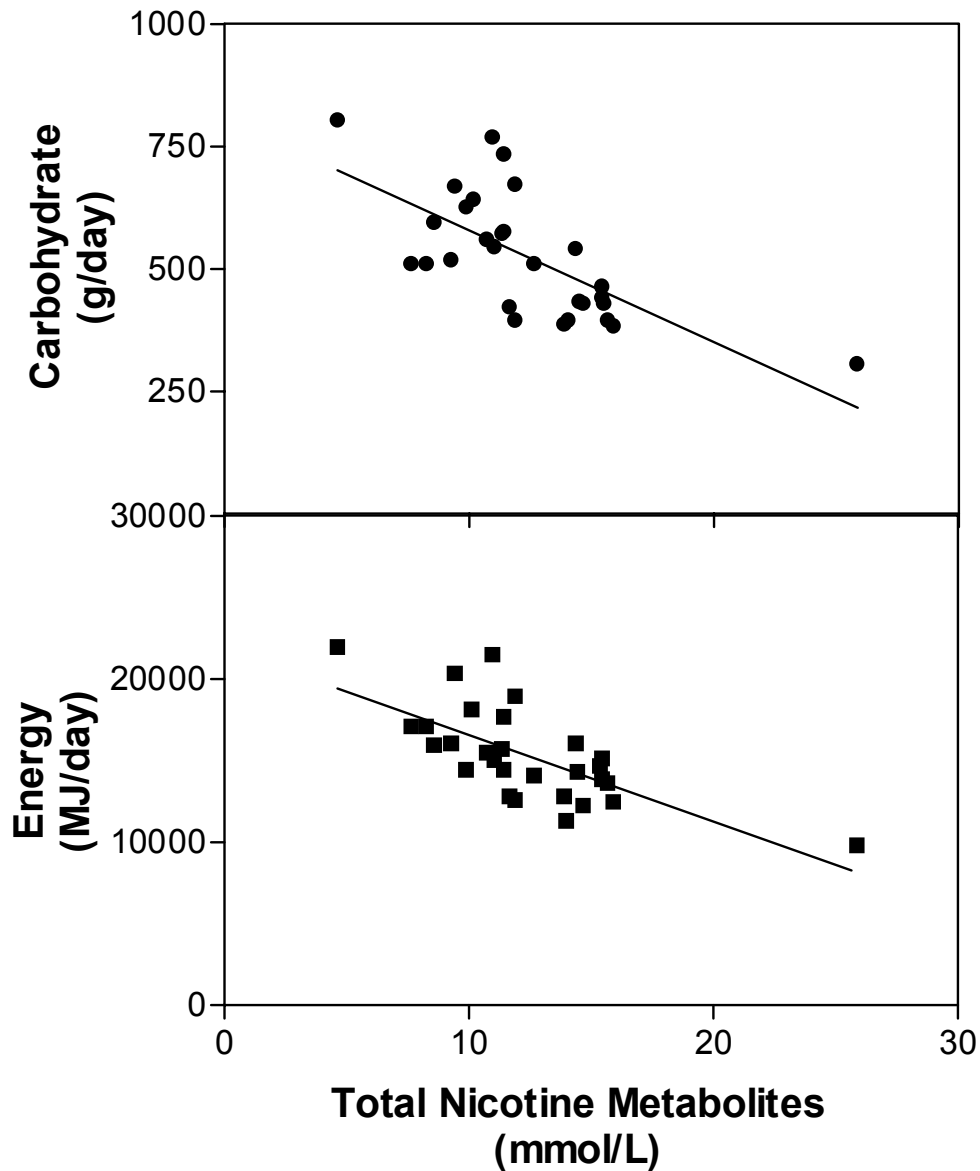


Figure 3 Linear regression analysis of mean daily dietary intake (energy and carbohydrate) versus mean concentration of total nicotine metabolites in urine recorded by all participants over 23 days ($n = 28$).

The mean energy consumption by sappers at THR and DVFC represented an estimated 90% of estimated TEE for sappers at THRS and between 75% and 105% of the estimated energy requirements for the two tasks at DVFC. For the THRS group, mean daily energy intake ranged from 10 MJ to 22 MJ, and for the DVFC group from 12 MJ to 17 MJ. Mean TEE for THRS trail clearing team was 18 MJ (range 12–20 MJ, one day

observation). Chain-sawing and load carriage tasks had the greatest estimated TEE. Mean TEE for tree platform construction at DVFC was 18 MJ (range 14–20 MJ, two days observation) and for the road survey task 13 MJ (range 11–16 MJ, two days observation). Construction team members who worked above ground on the tree platform had the greatest estimated energy expenditure (range 19–22 MJ, two days observation).

3.1.1 Acceptability of HooAh!™ bar and Ergo Drink™

As stated in the Introduction, the HooAh!™ bars used in this study were the first commercially-manufactured versions of this carbohydrate supplement. The scientists' initial impressions of the bars were that they were of poor quality when compared with the laboratory-manufactured product used in a previous study [5]. In particular, the texture of the latest products was unappealing and both sappers and scientists found the bars too sweet to eat on a regular basis. Participants who had eaten the original product reported liking the laboratory-manufactured peanut butter flavoured HooAh!™ bar 'very much', while the sappers in this study reported being neutral about all three flavours (raspberry, peanut butter and apple cinnamon) of commercially-manufactured bars.

The Ergo Drink™ received similar ratings in both field studies: 'like moderately' to 'slightly dislike'. Participants in both studies found the Ergo Drink™ difficult to prepare.

Few sappers felt that eating the HooAh!™ bar improved their energy level, while most felt that the Ergo Drink™ was beneficial.

3.2 Physical Performance

Sappers at THRS recorded reduced single jump power ($t = -2.545$, $p = 0.02$), no change in abdominal strength and endurance (sit-ups), improved hand-grip strength ($F = 7.128$, $p = 0.009$ for right arm) and improved aerobic capacity ('beep test') ($F = 12.986$, $p = 0.001$). The improvement in estimated aerobic capacity needs to be viewed in light of body mass losses in the range 0.3% to 9.6% (Section 3.1), because any improvement may be due to reduced body mass and not increased fitness. At DVFC, where only standing vertical jump and sit-up tests were performed, no change in either single jump power or abdominal strength and endurance was detected. Changes in physical performance tests were not sensitive to estimated dietary intake, however changed right hand-grip strength was positively correlated with change in body mass ($r = 0.445$, $p = 0.032$). Mean test results for each group are given in Table 2.

Table 2 Mean and SEM for fitness test results recorded at Tawau Hills Ranger Station and Danum Valley Ranger Station

	Pre-study	Day 3	Day 8	Day 10	Day 17	Day 23	Change
Tawau Hills Ranger Station							
VO _{2max} (mL/kg/min)		48.9 (1.0)		50.5 (1.3)		52.2 (1.0)	3.5 (0.7)
Standing vertical jump (cm)	47.3 (1.5)	45.8 (1.3)		43.1 (1.5)		45.3 (1.7)	-2.1 (0.9)
Right handgrip (kg)		50.3 (1.7)		51.5 (2.0)		54.4 (1.6)	6.7 (3.2)
Left handgrip (kg)		51.6 (1.6)		51.4 (1.7)		52.8 (1.8)	6.6 (4.1)
Sit ups (number in 2 min)	82 (4)	82 (4)		81 (4)		83 (4)	2 (2)
Danum Valley Field Centre							
Standing vertical jump (cm)	45.6 (2.2)	48.3 (1.6)	47 (1.6)	47.6 (1.8)	47.2 (1.6)	45.0 (1.2)	-1.3 (1.1)
Sit ups (number in 2 min)	70 (3)	69 (3)	73 (4)	75 (3)	74 (3)	71 (3)	4 (1)

3.3 Physiological Strain and Environmental Thermal Stress

During February and March Sabah was hot and very humid, with rain showers occurring most days. The nights at THRS, where sappers were located on the ridge of a mountain and accommodated in hammocks, were mild and perceived as cool to cold once physical activity ceased. The heat of the day caused most sappers to self-pace by taking frequent rests when working hard physically such as carrying heavy loads (often up hill) or constructing tree-top platforms. Appendix B includes the physiological measures of sappers involved in some typical expedition tasks.

Mean WBGT at THRS was 23.1°C, with a maximum of 25.7°C and a minimum of 19.2°C taken between 06:00 and 19:00. At DVFC, the mean WBGT was 26.8°C with a maximum of 34.0°C and a minimum of 22.0°C, and a maximum globe temperature (radiant load) of 50°C taken across the day. Hence sappers at DVFC worked for short periods under conditions of thermal stress.

No significant levels of cardiovascular strain were observed at either location, and at THRS, where core body temperatures were recorded, thermal strain was not excessive. Maximum core temperatures ranged from 37.9 to 38.7°C for sappers at THRS. Core

temperatures above 39 to 39.5°C are considered to indicate excessive thermal strain and high risk of heat-related illness.

There was a mean heart rate across all days of 101 bpm (range 55–85 bpm) at THRS and a mean of 97 bpm (range 60–180 bpm) at DVFC. The time series data showed that sappers at both sites paced themselves by resting and recovering when physiological strain (heart rate and body core temperature) became excessive.

At DVFC, where sappers recorded RPE, TS and TC, those working on the tree platform recorded exertion as *very hard* to *very, very hard*, while those working on the ground recorded exertion as *somewhat hard*. Tree construction teams found the temperature of their bodies to be *extremely hot* (TS) and *uncomfortable* (TC), while the road survey team perceived themselves to be *slightly warm* to *hot* (TS), which they considered *comfortable* to *slightly uncomfortable* (TC). The psychophysical responses do not reflect the observed thermal strain and may be indicative of poor acceptance of high skin wettedness. This poor perceived thermal tolerance is not unexpected in this population, considering their normal place of occupation (UK).

3.4 Hydration Status

Water delivery to sappers at THRS was difficult, because of the terrain. The river, which was the major water supply for the group, was close to the first campsite at the base of the mountain ridge and was 12 km from the final camp site high up on the mountain ridge. Water resupply at THRS was conducted daily by members of the trail (and scientific) teams who carried 20 L containers of water along the trail. Rain water was collected to supplement this water supply. Sappers at DVFC, who were accommodated at a base camp, had easier access to water and mostly carried a personal supply of water to work sites.

The relative difficulty in supplying water to the two groups appears to be reflected in urine osmolality (Fig 4) and SG measurements. Mean urine osmolality at THRS was 805 mmol/L (range 424–1,075 mmol/L) and 462 mmol/L (range 210–839 mmol/L) at DVFC. Hypohydration was common at THRS for the first three days, when nearly 50% of sappers had highly concentrated urines. Although urine measurements were not made regularly thereafter, the incidence of highly concentrated urine stabilised to no more than one or two sappers on any testing day at both locations.

Observational studies at both locations indicated that water consumption rates from 0.3 to 0.75 L per h provided adequate hydration (Appendix B). The mean water consumptions recorded by a trail team at THRS, a tree construction team at DVFC and a road survey team at DVFC were 0.42 L per h (range 0.3 to 0.75 L per h), 0.35 L per h (range 0.3–0.6 L per h) and 0.37 L per h (range 0.3–0.4 L per h), respectively. Mean sweat rates recorded by a tree construction team on two days were 0.33 L per h and 0.31 L per h and for a road survey team the mean was 0.2 L per h (both days). Only one participant (n = 22 individual observations) at THRS recorded an excessively

concentrated urine during these observational studies. These water consumption rates are less than those required to maintain hydration during a land mine clearance task (sweat rates of 0.47 to 0.63 L per h) and more arduous tasks such as pack loaded route marching or patrolling, all conducted in hot environments [16,17,18]. In the latter tasks water consumption of up to 1.5 L per h, thought to be the limit of human consumption, is recommended [17, 18].

Urine osmolality and SG measurements were highly correlated ($r = 0.97$), and using cut-offs of $\geq 1,000$ mmol/L for osmolality and ≥ 1.030 for SG both methods detected a similar incidence of hypohydration ($\chi^2 = 1393$, $p < 0.001$). Therefore the more robust method of measuring urine specific gravity is recommended for use in future studies.

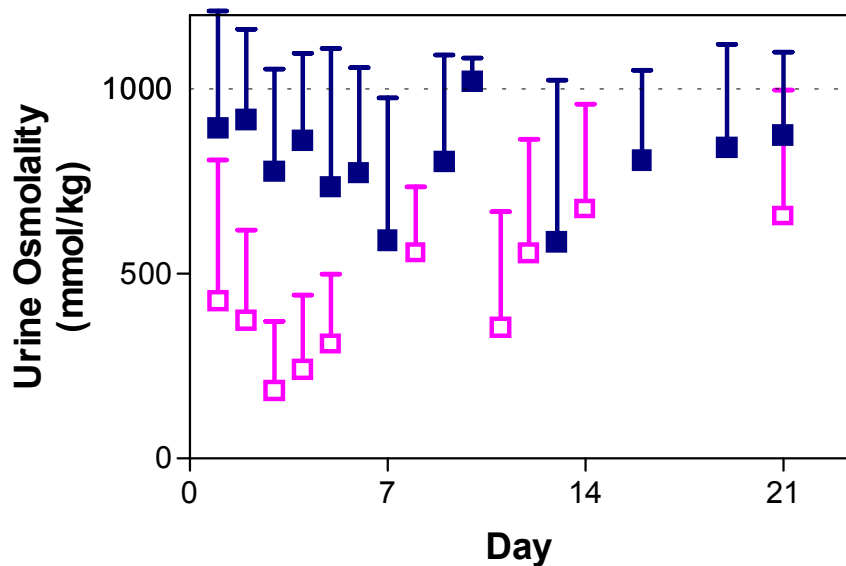


Figure 4 Mean and SD for urine osmolality recorded at Tawau Hill Ranger Station and Danum Valley Field Centre. Tawau Hills Ranger Station ■, Danum Valley Field Centre □.

3.5 Immune Function and Nutritional Status

At baseline the expedition group (n = 31) had good immune function and generally good nutritional status. However, some micronutrient deficiencies were detected, namely suboptimal plasma antioxidant capacity (25%, range 1.0–4.5 mmol/L), elevated PIVKA II (41%, range 0.23–4.84 ng/mL) and elevated Hcys (97%, range 3.2–141.6 μ mol/L), which are indicative of antioxidant nutrients (vitamin, minerals and endogenous non-food derived antioxidants), vitamin K and folic acid status. No biochemical deficiency of iron was evident and only two sappers had mild deficiency of riboflavin and vitamin B₆.

Sappers from both locations had good immune function before and after the study period. Cell-mediated immune function remained unchanged while some stimulation of humoral immune function (salivary sIgA:Alb) was detected ($F = 17.34$, $p < 0.001$, Fig 5). The trend for higher sIgA:Alb recorded at DVFC approached significance ($p = 0.054$). Mean results for plasma cytokines (IL-2, IL-6, IL2r) and skin hypersensitivity are given in Appendix C.

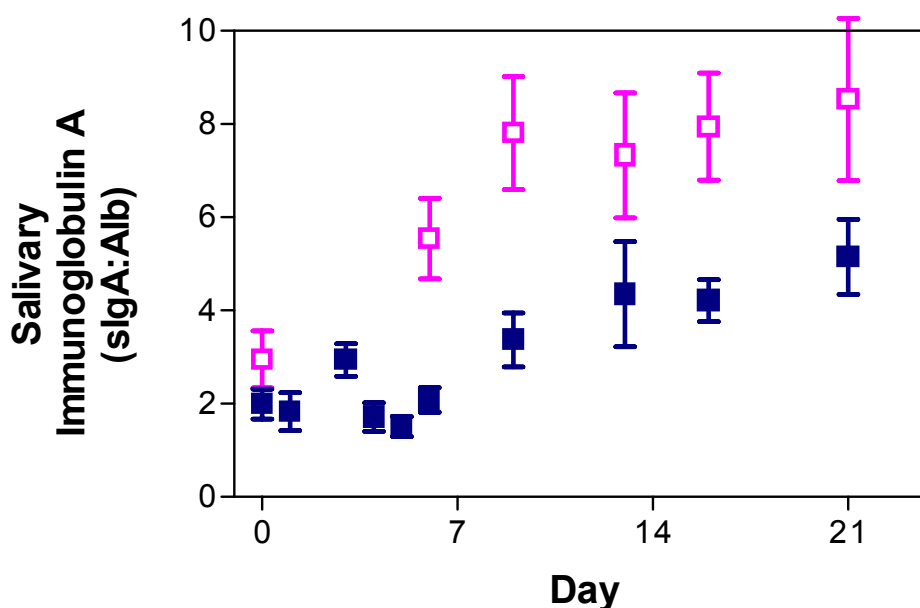


Figure 5 Mean and SEM salivary sIgA:Alb recorded for sappers at Tawau Hills Ranger Station and Danum Valley Field Centre. Tawau Hills Ranger Station ■, Danum Valley Field Centre □.

Sappers at both locations experienced mild metabolic stress with some loss of visceral protein stores (IGF-1 and fibronectin), oxidant stress and decreased iron stores. Sappers at both locations improved their riboflavin status. While the vitamin K status of sappers at DVFC improved and vitamin B₆ and folic acid status remained unchanged, sappers at THRS had unchanged vitamin K status and reduced vitamin B₆ and folic acid status at the end of the study (Table 3). Mean results for all biochemistry can be found in Appendix C. Table 4 describes the percentage of sappers who had micronutrient measures outside the healthy clinical ranges.

Table 3 Mean and SEM for biochemical changes recorded by sappers at Tawau Hills Ranger Station and Danum Valley Field Centre. Results of paired t tests are presented.

	Tawau Hill Ranger Station			Danum Valley Field Centre		
	% Change mean & (SEM)	t	p	% Change mean & (SEM)	t	p
TAOC	-21 (2.73)	-7.448	<0.001	-26 (0.4)	-3.035	0.002
IGF-1	-8.7 (5.8)	-1.958	0.065	-17.1 (4.1)	-4.767	0.001
PIVKA	22.8 (12.6)	0.741	0.468	-35.8 (12.1)	-2.422	0.046
Ferritin	-18.7 (4.1)	-4.467	<0.001	-21.01 (6.7)	-2.731	0.021
Fibronectin	-17.2 (5.7)	-3.446	0.003	-24.0 (9.4)	-2.62	0.028
Hcys	11.1 (10.4)	2.397	0.029	1.15 (9.4)	-0.373	0.717
EGRAC ^a	-30.9 (19.5)	-3.49	0.003	-51.2 (15.0)	-4.150	0.004
EASTAC	10.7 (3.3)	3.255	0.004	7.28 (3.53)	0.427	0.684

^a EGRAC is erythrocyte glutathione reductase activation coefficient and EASTAC is the erythrocyte aspartate transaminase activation coefficient.

Table 4 Proportion of sappers outside the optimal range for functional measures of micronutrient status.

	Clinical Cut-off	Proportion outside clinical range			
		Tawau Hills Ranger Station (n = 20)		Danum Valley Field Centre (n = 11)	
		First day	Last day	First day	Last day
TAOC	<1.2 mmol/L	40%	60%	0%	0%
PIVKA II	>2.0 µg/L	40%	50%	45%	36%
Ferritin	< 15 g/L	0%	5%	0%	0%
Hcys	> 10 µmol/L	95%	90%	100%	100%
EGRAC	> 40% activation	5% (1 participant)	5%	9% (1 subject)	0%
EASTAC	>120% activation	5%	0%	9%	0%

At THRS oxidant stress (or reduced antioxidant capacity of plasma) was negatively correlated with dietary intake ($r = -0.489, -0.523, -0.475$ and -0.426 for energy, protein, fat and carbohydrate respectively, all $p < 0.05$) and changes in plasma fibronectin and ferritin status were correlated with the change in aerobic capacity (Fig 6, $r = 0.653$ and

0.648 respectively, both $p < 0.002$). Alcohol consumption at DVFC was negatively correlated with changed iron (Fig 7), folic acid and riboflavin (measured as EGRAC) status ($r = -0.825, -0.674, 0.551$, respectively, all $p < 0.05$). Alcohol consumption was also negatively correlated with changed body composition (% body fat, $r = -0.684$, $p = 0.015$).

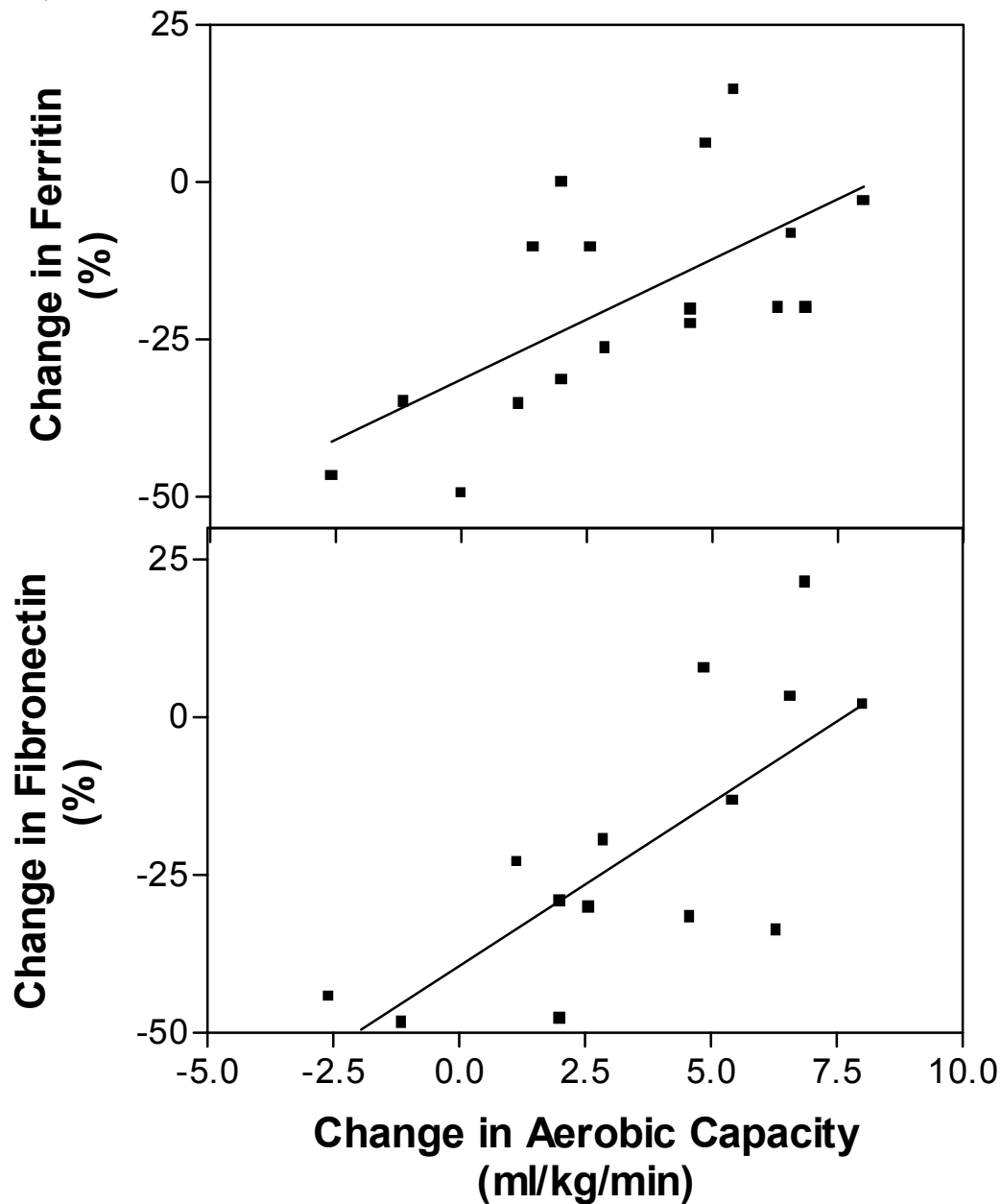


Figure 6 Linear regression analysis of change in plasma fibronectin and change in serum ferritin versus change in aerobic capacity recorded by sappers at Tawau Hills Ranger Station.

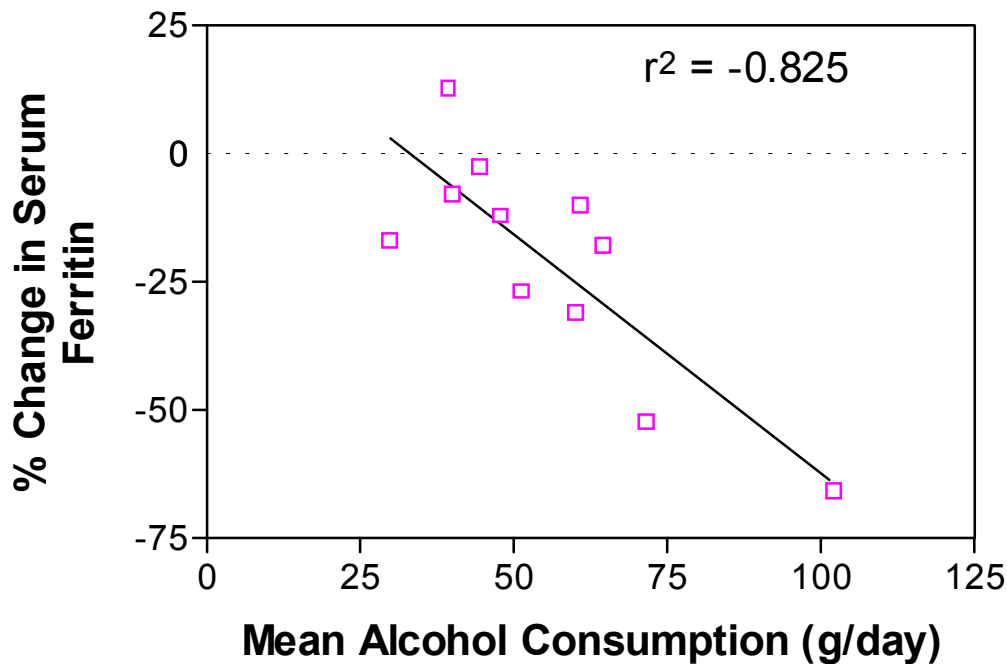


Figure 7 Linear regression analysis of changed serum ferritin versus mean alcohol consumption recorded by sappers at Danum Valley Field Centre.

3.6 Cognition, Fatigue, Mood and General Health

Apart from minor cuts, bruises, sunburn, insect and leech bites, few health problems were recorded. Although sappers at THRS recorded greater general health problems at the end of the study ($t = 3.390$, $p = 0.004$), this was only an increase from a mean score of 16.5 (SEM = 1.7) to 24.2 (SEM = 1.9) compared with a maximum possible score of 108. The general health problems recorded at DVFC remained stable throughout the study.

Despite increased *lack of energy* (THRS $t = 2.002$, $p = 0.061$, DVFC $t = 2.424$, $p = 0.036$) and decreased *vigour* ($F = 2.942$, $p = 0.043$) at both locations, and increased physical discomfort at THRS ($t = 3.601$, $p = 0.002$) sappers at both locations steadily improved on the ASA cognitive tests as the study progressed ($F = 18.406$, $p < 0.001$) and did not suffer from lack of motivation (Figs 8 and 9). The first day of constructing tree platforms, which involved hauling timber to the work site, corresponded with an acute increase in the POMS factor *fatigue* ($p = 0.046$) and an acute decrease in the POMS factor *vigour* ($p = 0.031$). Technical problems during the first several days of work at DVFC registered as an increase in the POMS factor *anger* ($F = 3.65$, $p = 0.019$).

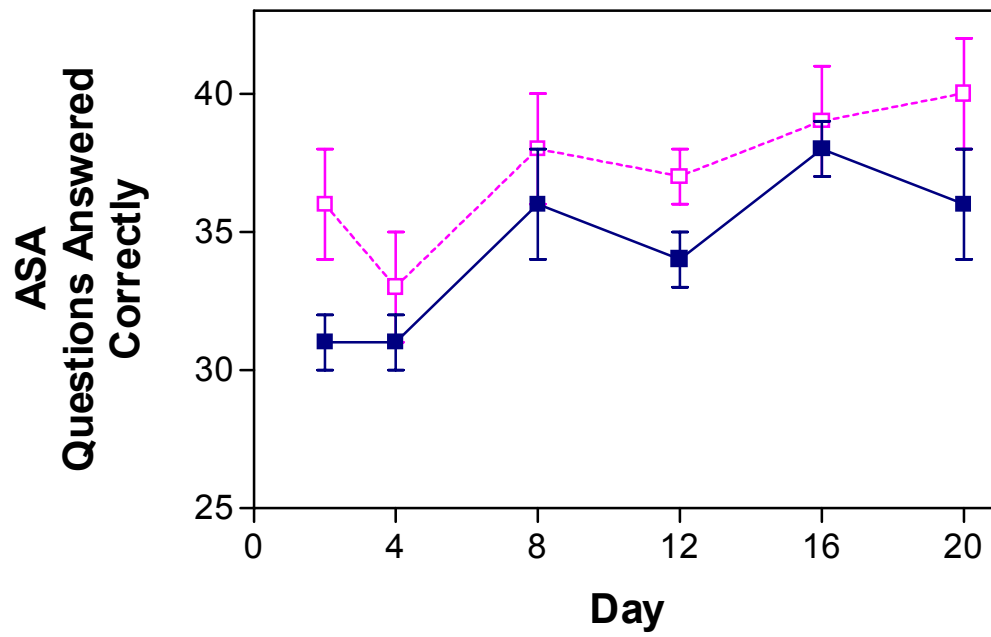


Figure 8 Mean and SEM for ASA test questions answered correctly by sappers at Tawau Hills Ranger Station and Danum Valley Field Centre. Tawau Hills Ranger Station ■, Danum Valley Field Centre □.

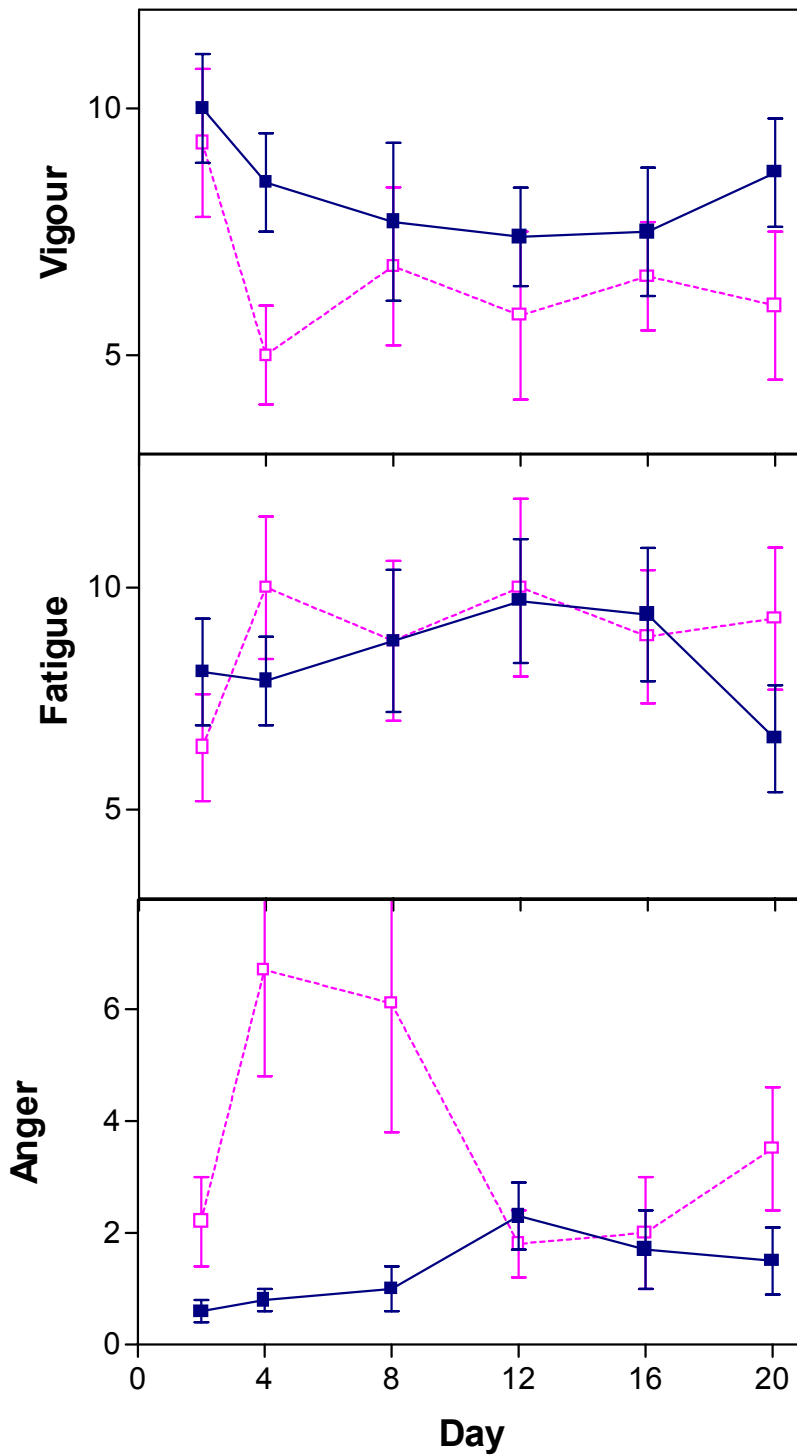


Figure 9 Mean and SEM for POMS factors vigour, fatigue and anger recorded by sappers at Tawau Hills Ranger Station and Danum Valley Field Centre. Tawau Hills Ranger Station ■, Danum Valley Field Centre □.

4. Discussion

4.1 Consumption of Combat Ration Packs

It is a common experience of military organisations around the world that soldiers tend to lose body mass during field operations. This exercise was no exception, with body mass losses ranging from less than 1% to over 9% (Section 3.1). However, the mean body mass loss of around 5% over three weeks recorded during this study would not be expected to cause a decrement in physical or mental performance, and this was found to be the case (Sections 3.2 and 3.6).

Rate of body mass loss in the well-hydrated soldier is important because it is primarily related to energy deficit. Body mass losses due to uncomplicated protein-energy malnutrition (PEM) of up to 10% over three weeks are not likely to have a detrimental effect on military performance, providing soldiers are able to regain body mass before redeployment [19]. Under-consumption of food can be a problem among rapidly re-deployed soldiers who have lost more than 5% of body mass without opportunity to regain body mass, because further body mass losses are likely to result in an overall loss of muscle and decreased military performance.

The principal cause of body mass loss in the well-hydrated soldier is a combination of under-consumption of food and very high energy expenditure. For example, ADF soldiers discard up to 30% of their CRP contents during operations [4,5]. Multiple logistical, situational and sensory factors contribute to under-consumption during field operations. Meal schedules, military and unit command emphasis and military feeding policy will affect food consumption, as will the soldier's perception of individual food acceptability and the opportunity to share meals. The fatalistic attitude of some commanders and soldiers towards body mass loss during field operations may contribute to under eating and often leads to deliberate dieting. Operational anorexia as a generalised stress response to severe environment, thermal strain, hypohydration, anxiety, fatigue, aches and pain will also reduce food consumption [20].

EX PF presented an opportunity to test the acceptability of Australian CR1M under field conditions, without the complications caused by the usual operational stresses. The CR1M provides about 14 MJ. On average, the sappers at THRS consumed 16 ± 4 MJ/day (about 90% of their mean estimated TEE/day) and 560 ± 130 g carbohydrate/day. Despite the low level of consumption of the carbohydrate supplements (Ergo™ and HooAh!™), this level of food intake corresponds to eating slightly more than one CR1M per person per day. This is a higher consumption rate than previously observed for ADF soldiers rationed with CRP under field conditions.

The psychological testing indicated that the sappers remained mentally alert and were in good health, good mood and were only mildly fatigued despite the harsh environmental conditions. This would most likely be due to the nature of the training

exercise. For example, although the participants worked hard, were under time constraints to finish their tasks, and experienced some frustration in the completion of their work, they were able to socialise, take meal breaks and have sufficient sleep. An additional factor in promoting consumption of CRP at THRS was the fact that the rations were cached and freely available. Sappers tended to pull the packs apart, taking components for snacks and individual meals as required for the day's work.

Participants in this study may have eaten well because of the novelty of being able to eat a variety of foods with which they were not familiar – Australian CRP items, US Army carbohydrate supplements and local Malaysian foods. Often there is a heightened acceptance of foods when they are eaten for the first time. However, although there is a general perception that monotony reduces consumption, there has been little military research to address this issue [21]. Hedonic ratings represent only one aspect of a complex issue, for example certain foods such as bread and milk, which usually receive an average hedonic rating, will continue to be consumed in large quantities well after other more highly rated foods have been rejected as no longer being palatable.

Hypohydration has been associated with reduced appetite and food consumption [22]. Furthermore, there is a suggestion that when individuals expect an extended period of limited water consumption they may reduce their food intake even if hypohydration is not yet evident. Because few sappers experienced hypohydration (Section 3.4), water availability and consumption does not appear to have been a factor in under-consumption of food during this study.

4.2 Nutritional Status

There is evidence of mild PEM and micronutrient deficiency for sappers at both THRS and DVFC (Section 3.5). The micronutrients that were found to be limiting for both groups were iron and antioxidant nutrients. In addition, the CR1M diet was found limiting for folic acid and vitamin B₆. As discussed in a previous report [5] the measure of antioxidant status used in this study cannot distinguish between the various antioxidants (e.g. vitamin C, vitamin E, carotenoids, organoselenium) so it cannot be determined if a particular micronutrient(s) was limiting. These findings are consistent with those of a previous study involving CR1M [5] where under-consumption of the ration and consequent PEM were worse. In the present study PEM was not sufficient to adversely affect immune function, cognition or physical performance. Furthermore, because there was no evidence of loss of lean body mass or muscular strength, the dietary intake of carbohydrate (560 ± 130 g/day at THRS, 430 ± 140 g/day at DVFC) appears to have been sufficient to prevent any significant loss of muscle protein.

Although the decline in micronutrient status was not accompanied by observable immune suppression or ill-health, it remains a problem which should be addressed, because repeated deployment of soldiers without sufficient nutritional recovery could lead to depletion of micronutrient stores. Prospective data on the micronutrient status

of repeatedly deployed soldiers is not available, but a negative inference may be drawn from the apparently poor micronutrient status of this cohort of British soldiers (Table 4) and that of an Australian cohort [5]. Good stores of micronutrients are needed for maintenance of acute health such as optimal immune function [23] and for prevention of chronic health problems in later life, particularly cardiovascular disease [24, 25]. As an example, epidemiological evidence indicates that iron status plays an important role in determining susceptibility to and severity of infections [26].

The trend for reduced iron stores found in a previous, medium-term CR1M study [5] was found to be even greater at the completion of the present, longer study. Because the former study involved more pronounced PEM, it may be that a combination of under-consumption, prolonged moderate to high physical activity and environmental conditions adversely affects iron status. Although only one individual had clinical iron deficiency on completion of EX PF, the decline in iron status is of concern, because iron deficiency anaemia can be expected to have adverse effects on military performance. Furthermore, the estimated aerobic capacity of sappers at THRS correlated with their iron status (Fig 6). This is consistent with controlled studies in which poor iron status has been shown to compromise performance during prolonged exercise such as long-distance running [20]. The US Committee on Military Nutrition Research recommends that personnel with iron deficiency should receive appropriate medical treatment and monitoring until laboratory results show a return to normal values, and also that those with iron deficiency should not be deployed [27].

A further concern was the adverse effects of alcohol consumption and tobacco smoking on food consumption and nutritional status (Sections 3.1 and 3.5). The effects of tobacco smoking were consistent with the findings of the previous CR1M study – those who smoked most tended to eat the least food, particularly carbohydrate-rich foods [5]. In the previous study, tobacco smoking was also correlated with urine specific gravity (i.e. it was a predictor of hypohydration).

Sappers at DVFC had access to beer with their evening meal on six occasions and drank a mean of ~1,900 mL (~ 75 g ethanol) per occasion (Table 1). This corresponds to ~7.5 standard drinks. It appears that this beer may have displaced some of the available food, as indicated by a reduced micronutrient status associated with beer consumption. The negative association between alcohol consumption and serum ferritin concentration is of particular concern for reasons outlined above (Fig 7). Although not found to be the case in this study, alcohol consumption during a RAAF survival school course was associated with reduced humoral immune function [28].

4.3 Testing Methods

Field assessment of physical performance, cognition, dietary intake, and physiological and nutritional status is challenging. This was particularly so during the present study, where both sappers and scientists worked hard under harsh environmental conditions.

Testing protocols were limited by practical considerations, particularly the need for international transport of equipment and biological samples, a small research team, the requirement for load carriage by scientists at the study sites, storage of biological samples and limited access to electrical power at one of the sites. A report detailing the strengths and weaknesses of the study, and making recommendations for future studies, is available from (christine.booth@defence.gov.au) [29].

Based on the experience of EX PF and the study results, methodologies for assessment of physical performance, dietary intake and some of the biochemical measures such as antioxidant status and immune status require further development. Measures of physical performance are variously affected by loss of body mass. For example, decrements in US Rangers' performance were well demonstrated by a maximal lift capacity test, whereas hand-grip strength was insensitive to loss of body mass [30].

Physical performance tested during prolonged continuous effort such as jogging can give different results to acute tests of muscle strength. An estimation of aerobic capacity by use of the beep test is probably a reliable and practical indicator of changes in physical performance, but even these results need to be interpreted with caution (Section 3.2). Data from a biomarker of skeletal muscle turnover (e.g. 3-methylhistidine) would assist in the interpretation of physical performance data [5].

Collection and analysis of dietary intake data is critical to understanding decrements in military performance during field operations, but is the most difficult and labour-intensive aspect of field study. Consideration needs to be given to refining dietary intake and analysis methods to make optimum use of electronic data entry and handling. Biochemical assessment of antioxidant status and genotoxic stress could be improved by use of biomarkers of lipid oxidation and DNA damage, and more frequent monitoring of immune function could be achieved by use of biomarkers in urine (e.g. biopterin and IL6) and saliva (e.g. sIgA, biopterin).

5. Conclusions

1. Most sappers nearly matched their energy intake and expenditure, and the body mass losses experienced were unlikely to have a detrimental effect on military performance. Factors which may have encouraged food consumption included the novelty of eating unfamiliar foods, ability to socialise and take meal breaks, ability to self-select food items and number of serves, adequate sleep, good morale, and good hydration status.
2. To meet the energy requirements of an engineering task involving prolonged moderately-vigorous physical activity, a group of British sappers needed to eat more than the contents of one CR1M per day.
3. Under the conditions of this adventurous training exercise, where soldiers were able to consume more than one ration pack per day, the CR1M provided sufficient energy and macronutrients to prevent serious protein energy malnutrition for up to 23 days of moderate-to-high physical activity in a hot environment, but failed to provide sufficient iron, folic acid, antioxidants and vitamin B₆ to prevent a decline in storage of these nutrients.
4. Tobacco smoking and, when available, alcohol consumption contributed to under-consumption of food during this field exercise.
5. Alcohol consumption may have a particular negative effect on iron status.

6. Recommendations

1. Further research is required to investigate the causes of operational anorexia, including the effects of:
 - hydration status;
 - food monotony; and
 - training command emphasis, field feeding policy and attitudes of individual commanders.
2. An additional long-term field study of nutrition under realistic training conditions is required to assess the effects of operational anorexia on health and military performance, and to determine the most appropriate strategies for ensuring that adequate recovery has occurred before soldiers are redeployed.
3. The CR1M should be assessed for adequate micronutrient content, in particular folic acid, vitamin K, iron, antioxidant nutrients and vitamin B₆. This can be achieved by a combination of laboratory work (chemical analysis and

controlled human studies) and field work (nutritional survey and field studies, as suggested above).

4. The phenomenon of declining iron stores during periods of prolonged moderate to high physical activity in a tropical environment needs further investigation. In particular, the possible implications for redeployment of soldiers need to be determined.
5. The potential for tobacco smoking and alcohol consumption to adversely affect military performance needs further investigation.
6. Testing methodologies for the study of soldier performance during sustained operations needs further research.

7. Acknowledgements

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8. References

1. Department of Defence (2000). *Defence 2000, Our Future Defence Force* (the White Paper). Commonwealth of Australia © 2000. ISBN 0 642 295441.
2. AMMA. (1997). *Australian Defence Force Ration Scales and Scales of Issue (SUPMAN 4)*, Edition 5. Army Material Management Agency Support Command Australia (Army), Directorate of Publishing, Defence Centre, Canberra.
3. Forbes-Ewan, C. (2000). *Revision of Australian Combat Ration Packs*. DSTO, Defence Nutrition Research Centre, Scottsdale, Tas 7260
4. Forbes-Ewan C. and Waters, D. (2000). *Final Report of TTCP-HUM-AG16*. DSTO, Defence Nutrition Research Centre, Scottsdale, Tas 7260
5. Booth, C., Coad, R., Forbes-Ewan, C. Thomson, G. and Niro, P. (2001). *The Effect of Consumption of Australian Combat Rations on Military Personnel after a Medium-Term Field Exercise*. DSTO-RR-0228 Combatant Protection and Nutrition Branch, AMRL, Melbourne, VIC.
6. Workman, M. (2000). *Sabah 2000: Exercise Pelopor Finn – the Collaboration of Multidisciplinary Scientific Research and British Military Training, an Assessment*. Exercise Pelopor Finn 1999–2000 Post Expedition Report 25 Engineer Regiment\Corps of Royal Engineers (UK).
7. Lukaski H., Johnson, P., Bolonchuk, W. and Lykken, G. (1985). Assessment of fat-free mass using bioelectrical impedance measurements of the human body, *Am. J. Clin. Nutr.* 41:810-817.
8. Defence Nutrition Research Centre (2001). Bioimpedance analysis. DNRC Method ID 897/1424, version 1.2.
9. Brewer, J., Ramsbottom, R. and Williams, C. (1988). *Multistage Fitness Test. A Progressive Shuttle-Run Test for the Prediction of Maximum Oxygen Uptake*. Australian Coaching Council, Belconnen, ACT.
10. Walmsley, R.N. & White, G.H. (1994). *A Guide to Diagnostic Clinical Chemistry*, pp270-271, Blackwell Scientific Publications, Melbourne, VIC.
11. Pickert, A., Lingenfeller, T., Pickert, C., Birbaumer, N., Overkamp, D., Eggstein, M.(1993). Comparison of a mechanised version of the 'König' reaction and a fluorescence polarisation immunoassay for the determination of nicotine metabolites in urine, *Clin. Chim. Acta.* 217:143-152.

12. American College of Sports Medicine. (2000). *ACSM's Guidelines for Exercise Testing and Prescription*, Sixth Edition, Lippincot, Williams & Wilkins, Philadelphia, USA.
13. Gagge, A.P. and Hardy, J.D. (1967). Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Environ. Res.* 1:1-20.
14. Ainsworth, E., Haskell, W., Leon, A., Jacobs, D., Montoye, H., Sallis, J. and Paffenbarger, R. (1993). Compendium of physical activities: Classification of energy costs of human physical activities. *Med. Sci. Sports. Exerc.* 25 (1):71-80.
15. Allena, J., Savon S., Jacobsen, D. (1995). Determination of total serum sulfite by HPLC with fluorescence detection, *Clin. Chem.* 41:897-903.
16. Amos, D., Cotter, J.D., Lau, W.M and Forbes-Ewan, C.H. (1998). *A Methodology for Measuring the Physiological Strain of Enhanced Soldiers: The 1998 Soldier Combat System Enhancement Study*. DSTO-TR-0747, AMRL, Melbourne.
17. Cotter, J.D., Roberts, W.S., Amos, D., Lau, W.M. and Prigg, S.K. (2000). *Soldier Performance and Heat Strain During Evaluation of Combat Fitness Assessment in Northern Australia*. DSTO-TR-1023, AMRL, Melbourne.
18. Lau, W.M., Roberts, W.S. and Forbes-Ewan, C. (1999). *Physiological Performance of Soldiers Conducting Long Range Surveillance and Reconnaissance in Hot, Dry Environments*. DSTO-TR-0894, AMRL, Melbourne.
19. Committee on Military Nutrition Research. Food and Nutrition Board, Institute of Medicine (1999). *Military Strategies for Sustainment of Nutrition and Immune Function in the Field*, p 7, National Academy Press, Washington DC, USA.
20. Committee on Military Nutrition Research. Food and Nutrition Board, Institute of Medicine (1995). *Not Eating Enough. Overcoming Under-consumption of Military Operational Rations*, pp 10-18, Bernadette M. Marriott, Ed, National Academy Press, Washington DC, USA.
21. Committee on Military Nutrition Research. Food and Nutrition Board, Institute of Medicine (1995). *Not Eating Enough. Overcoming Under-consumption of Military Operational Rations*, p 211, Bernadette M. Marriott, Ed, National Academy Press, Washington DC, USA.
22. Committee on Military Nutrition Research. Food and Nutrition Board, Institute of Medicine (1995). *Not Eating Enough. Overcoming Under-consumption of Military Operational Rations*, pp 222-226, Bernadette M. Marriott, Ed, National Academy Press, Washington DC, USA.

23. Rall, C. and Meydani, N. (1993). Vitamin B6 and immune competence. *Nutr. Revs.* 51(8):217-224.
24. Boushey C., Beresford, S., Omenn, G. and Motulsky, A. (1995). A quantitative assessment of plasma homocysteine as a risk factor for vascular disease - probable benefits of increasing folic acid intakes. *J.A.M.A.* 274(13):1049-1057.
25. Braam L., Dissel, P., Gijsbers, B., Spronk, H., Hamulyak, K., Soute, B., Debie, W. and Vermeer, C. (2000). Assay for human matrix Gla protein in serum - Potential applications in the cardiovascular field. *Arteriosclerosis Thrombosis & Vascular Biology* 20(5):1257-1261.
26. Scrimshaw, N. and San Giovanni, J. (1997). Synergism of nutrition, infection, and immunity: an overview. *Am.J.Clin.Nutr.* 66:464S-477S.
27. Committee on Military Nutrition Research. Food and Nutrition Board, Institute of Medicine (1999). *Military Strategies for Sustainment of Nutrition and Immune Function in the Field*, pp 60-63, National Academy Press, Washington DC, USA.
28. Carins, J. and Booth, C. (2001). *Evaluation of a Simple Immunological Test (sIgA) During the RAAF Survival Course*. DSTO-RR-0224 AMRL, Melbourne.
29. Booth, C. (2000). *Post Activity Report: Exercise Pelopor Finn*. DSTO DNRC file 432/24/25b (64), Scottsdale.
30. Shippee, R., Friedl, K., Kramer, T., Mays, M., Popp, K., Askew, E., Fairbrother, B., Hoyt, R., Vogel, J., Marchitelli, L., Frykman, P., Martinez-Lopez, L., Bernton, E., Kramer, M., Tulley, R., Rood, J., Delany, J., Jezior, D. and Arsenault, J. (1994). *Nutritional and Immunological Assessment of Ranger Students with Increased Caloric Intake*. U.S. Army Research Institute of Environmental Medicine Report T95-5, Natick MA 0176-5007.

Appendix A: Information and Consent Forms

A.1. CONSENT FORM: NUTRITION & PHYSIOLOGY STUDY EXERCISE PELOPOR FINN

Task No: ARM 98/099

I..... give my consent to participate in the study mentioned above on the following basis:

- I have had explained to me the aim of this research, how it will be conducted and my role in it. I am happy to participate.

I understand that I have agreed to have my mental, physical and nutritional status measured. This involves psychological questionnaires, fitness tests, donation of two fasting blood samples (30 mL each), measurement of core body temperature by use of a low-frequency radio pill, two skin hypersensitivity tests, recording of food intake and collection of urine (~50 mL) and saliva (~2 mL) samples on most days. I understand that the protocol of taking and handling my blood samples will conform to conventional medical practice and that the risk to myself of a deleterious outcome will be no higher than for a routine medical examination. Health problems I have during the nutrition study will be recorded.

I have agreed to eat either freshly-prepared meals or Australian combat rations for the duration of the nutrition study (up to 24 days).

I understand that blood tests will include measures of vitamin status (folic acid, thiamin, riboflavin, total antioxidants, vitamin K, vitamin B6, homocysteine, F2 α isoprostane), protein status (fibronectin, insulin-like growth factor), and immune function (interleukin). Urine tests will measure hydration status (osmolality, specific gravity and electrolytes), labelled water components, amount of tobacco smoking (nicotine products), oxidative stress (8-hydroxy-2'-deoxyguanosine) and muscle protein turn-over (3-methylhistidine, 1-methylhistidine, creatinine). Saliva tests will measure IgA, albumin, cortisol and neopterin.

A.2. INFORMATION SHEET: NUTRITION & PHYSIOLOGY STUDY EXERCISE PELOPOR FINN

Task No: ARM 98/099

There have been few field evaluations of combat rations, even internationally. In fact Australian combat rations haven't been put-to-the-test since the 1960's.

Soldiers participating in a 34-day American trial experienced weight loss and a 23% decrease in lifting strength and in another American trial a dramatic suppression of immune function coinciding with increased rate of infection was reported. Military effectiveness will be compromised when effects such as these are experienced.

Nutrition is essential for soldiers to be able to maintain optimal physical performance in the field. Studies like this provide data on the operational effectiveness of combat rations and enable scientists to continually improve ration pack design. The ABCA agreement to make materiel as interchangeable as possible between nations involves America, Britain, Canada and Australia. An additional benefit of the study is that it will provide initial data on how well-accepted rations are between ABCA nations.

1. A nutrition study, which measures heat stress, hydration, nutritional status, physical fitness and mental state will be conducted during Exercise Pelopor Finn in February 2000. The study will be conducted over 24 days, which covers the climb of Mount Kinabalu and the nutrition study period. On most of these 24 days, your involvement will be 15 to 30 minutes. On the first and final days of the study in Malaysia your involvement will be 2 – three hours and during the training phase in the UK you will be required for several test periods of up to three hours duration.
2. You will be allocated to either the control or test group. The control group will receive freshly prepared meals and a ration supplement while the test group will receive Australian combat rations. You will also receive an experimental carbohydrate supplement.
3. You will be asked to complete some psychology questionnaires, which assess your mental ability, mood state and personality traits. These forms will be handed out at various times during the training period and during the study in Malaysia.
4. Before departure from the UK and again on returning to the UK your fitness level and body composition will be determined. See below for a detailed description of the fitness test. The fitness test may require up to 5 finger prick blood samples. If you feel that you cannot continue with any test, you will be able to stop immediately. Because some soldiers can push themselves beyond exhaustion, medical monitors will also stop your participation if signs of heat

exhaustion or stress are detected from you. Your body composition will be determined by underwater weighing.

5. Before commencement and again on the final day of the study in Malaysia you will be asked to perform a military fitness test and to donate a fasting blood sample (30 mL).

A field test of aerobic fitness (2.4 km run), will be undertaken in Malaysia in addition to the fitness testing conducted in the UK. Upper body strength will be assessed by performing sit-ups and flexed arm hang to volitional exhaustion or chin-ups to volitional exhaustion. Hand-grip will be determined using a hand-grip dynamometer.

You will be asked to consume no food for approximately 12 hours before the 'fasting' blood sample is collected. Usually this means not eating food from 2200 hours the night before donating the blood sample. The risk to you of deleterious outcomes during and/or after the study will be no higher than for routine medical examination. The blood collector will place a tourniquet around your upper arm then draw a blood sample from the inside of your arm into a syringe. Apart from a small prick when the needle pierces the skin, little discomfort is experienced by most people. However, if the procedure makes you feel faint, you should remain sitting and place your head between your knees. By applying pressure on the puncture site after the needle is withdrawn, bruising should be prevented. The use of sterile technique by the blood collector will prevent the risk of infection. Blood sampling by fingerprick will be performed in a controlled laboratory situation with appropriate infection control. Apart from some local pain (sting) and possible bruising, risk of side-effects from fingerprick blood sampling is minimal.

Blood tests will include measures of vitamin status (folic acid, thiamin, riboflavin, total antioxidants, vitamin K, vitamin B6, homocysteine, F₂isoprostane), protein status (fibronectin, insulin-like growth factor), and immune function (interleukins).

A skin test for cellular hypersensitivity, CMI Multitest, will be performed in conjunction with the collection of blood samples. The CMI Multitest is a disposable, plastic applicator consisting of eight sterile test heads preloaded with seven test antigens (tetanus toxoid, diphtheria toxoid, streptococcus, tuberculin old, candida, trichophyton and proteus antigens) and a glycerin negative control for percutaneous administration. A small amount of soluble antigen is introduced into the skin by puncture. Circulating T-cells (lymphocytes) sensitised to the antigen from prior contact, react with the antigens in the skin and induce a specific immune response, which manifests as a small red inflammation. The red area (2 - 10 mm) is measured 48 hours after application.

Blood collection and skin testing (CMI Multitest) will be conducted in a clean safe environment under the supervision of the expedition medical officer. Blood will be collected by suitably qualified staff, only. If you have ever had a severe allergic reaction

you must inform staff before receiving the skin test. In this case, you will not be skin tested.

All staff involved in handling blood, urine or saliva samples have been trained in correct safety precautions (Biohazard Level 2) for collection, processing and disposal of human tissue.

In order to determine your energy expenditure, you will be given either a 'labelled' or plain water sample. Naturally occurring water consists of Hydrogen and Oxygen atoms with a range of slightly differing atomic weights (or isotopes). The 'labelled' water has been specially purified to contain specific stable isotopes. Stable isotopes are NOT radioactive. This is a commonly used procedure for measuring energy expenditure in adults, children and newborn babies.

You will be asked to provide urine and saliva samples on several occasions during the training phase and again during and after the nutrition study in Malaysia. Urine samples will be used to determine muscle turnover (3-methylhistidine, 1-methylhistidine, creatinine, Nitrogen-15), energy expenditure (labelled water components), hydration status (osmolality, specific gravity and electrolytes), oxidative damage (8-hydroxy-2'-deoxyguanosine) and markers of tobacco smoking (nicotine metabolites). Saliva samples will be used to determine a stress hormone (cortisol) and markers of immune function (neopterin and IgA).

All aspects of the nutrition study will be conducted whilst you are 'on duty'. A trained data collector will assist you in the completion of the survey forms. After analysis of the data you will be provided with feedback on dietary information as well as blood chemistry, psychological and fitness status.

Your participation in this research is voluntary. You are free to withdraw from the study at any time without detriment to your military career or future medical care. The information collected will be kept confidential and nothing will be published which will identify individual participants. The information will only be used for this nutrition study.

Should you have any complaints or concerns about the manner in which this research is conducted, please do not hesitate to contact the chief investigator:

Dr Christine Booth, DSTO-Defence Nutrition Research Centre
76 George St, Scottsdale TAS 7620
Ph : 61 3 6352 2033, Fax: 61 3 6352 3044; E-mail: christine.booth@dsto.defence.gov.au
OR you may contact the Australian Defence Medical Ethics Committee:

Executive Secretary
Australian Defence Medical Ethics Committee
CP4-6-45
CANBERRA ACT 2600
Ph : 61 2 6266 3925, DNATS 8663925
Fax: 61 2 6266 4982, DNATS 8664982
E-mail: hlthpol@bigfoot.com

FITNESS TEST:

Maximal oxygen uptake (VO_{2max}) will be measured on a treadmill using open circuit spirometry. VO_{2max} is regarded as the best all-round measure of the capacity to engage in sustained physical work. The aerobic test is a standard VO_2 max test as routinely performed for testing athletes. The test involves the following procedure:

Subjects will be fitted with an external 'Polar' heart rate monitor to monitor heart rate throughout the test. Subjects will first warm up by walking on the treadmill for 5 minutes at 5-6 km/h. The VO_2 Max test will begin at a light run on the treadmill with the speed being increased at set time intervals. Once a maximum speed of 18 km/h for males has been reached, if the subject is happy to continue, the incline of the treadmill will then be increased. The incline will continue to be increased at 1 min intervals until the subject reaches exhaustion. Throughout the duration of the test the subject will breathe into a mouthpiece held steady on the head by a head piece. A nose clip will ensure mouth breathing. The subject is free to hit the emergency stop button on the treadmill at any time during the test should they want to stop for any reason. After the test is completed the mouthpiece and nose clip will be removed and the subject will walk on the treadmill for approximately 5 min to warm down.

Blood lactate levels are monitored by finger prick samples. Two to five finger prick samples will be taken over the duration of the test. Under most circumstances this will be two to three samples. The treadmill will be momentarily stopped for safety reasons while the blood is being sampled.

Appendix B: Physiological data

THTT = Tawau Hills trail team, DVCT1 = Danum Valley construction team 1, GPS = Survey team, Danum Valley

ID	Group	Day	max RPE	max TS	max TD	WBGT average 0C	Hrt rate average	Hrt rate range (bpm)	Body T average	Body T range	water cons L/hr	Sweat rate L/hr	total Ur loss (L)
4	THTT	13	NA	NA	NA	23.85	112	71	37.52	1.75	0.45	NA	0.15
14	THTT	13	NA	NA	NA	23.85	96	49	37.38	1.72	0.6	NA	0.35
27	THTT	13	NA	NA	NA	23.85	92	115	37.29	1.02	0.3	NA	0.04
18	THTT	13	NA	NA	NA	23.85	92	94	37.61	1.43	0.45	NA	0.04
6	THTT	13	NA	NA	NA	23.85	97	107	37.69	1.45	0.3	NA	0.09
20	THTT	13	NA	NA	NA	23.85	114	111	37.41	3.36	0.75	NA	0
26	THTT	13	NA	NA	NA	23.85	120	92	NA	NA	0.3	NA	0.07
34	THTT	13	NA	NA	NA	23.85	94	84	NA	NA	0.19	NA	0
Means						23.85	102	90	37.48	1.79	0.42		0.1
1	DVCT1	18	19	11	5	NA	105	98	NA	NA	0.4	0.35	1.2
12	DVCT1	18	17	11	3	NA	NA	NA	NA	NA	0.4	0.3	0.85
Means			18	11	4		105	98			0.4	0.33	1
5	GPS	18	NA	NA	NA	NA	90	124	NA	NA	0.41	NA	NA
16	GPS	18	NA	NA	NA	NA	92	99	NA	NA	0.41	NA	NA
Means							91	111			0.41		
1	DVCT2	15	13	11	3	29.74	96	91	NA	NA	0.34	0.03	2.5
2	DVCT2	15	15	10	3	29.74	93	74	NA	NA	0.3	0.02	1.6
24	DVCT2	15	13	11	3	29.74	99	99	NA	NA	0.2	0.05	1.7
25	DVCT2	15	13	11	3	29.74	100	94	NA	NA	0.6	1.4	0.39
12	DVCT2	15	18.5	11	4	29.74	103	109	NA	NA	0.4	0.14	1.4
32	DVCT2	15	18	12	4	29.74	87	84	NA	NA	0.25	0.23	0.47
Means			15	11	3.5	29.74	96	92			0.35	0.31	1.3
7	GPS	11	NA	9	2	25.46	92	119	NA	NA	0.32	0.14	1.3
5	GPS	11	NA	9	2	25.46	95	105	NA	NA	0.43	0.26	1.8
16	GPS	11	NA	8	1	25.46	87	50	NA	NA	0.35	0.17	2.1
8	GPS	11	NA	8	2	25.46	86	75	NA	NA			
Means				8.5	1.75	25.46	90	87			0.37	0.19	1.7

ID	Group	Day	TEE (MJ)	E cons (avMJ)
4	THTT	13	19.98	16
14	THTT	13	19.86	15
27	THTT	13	17.62	15.5
18	THTT	13	18.09	11.3
6	THTT	13	22.18	17.6
20	THTT	13	16.04	18.1
26	THTT	13	12	14.4
34	THTT	13	15.31	20.2
Means			17.6	16
1	DVCT1	18	19.45	12.4
12	DVCT1	18	22.4	13.6
Means			20.9	13
5	GPS	18	12.7	14.2
16	GPS	18	10.95	12.8
Means			11.8	13.5
1	DVCT2	15	18.5	12.4
2	DVCT2	15	13.5	14.2
24	DVCT2	15	18.1	14.3
25	DVCT2	15	19.3	12.8
12	DVCT2	15	18.5	13.6
32	DVCT2	15	22.4	15.1
Means			18.4	13.7
7	GPS	11	13.8	17.1
5	GPS	11	14.7	11.3
16	GPS	11	15.6	12.8
8	GPS	11	12.2	14.7
Means			14.1	14

Appendix C: Descriptive statistics for biochemical measures

C.1. Group at Tawau Hills Ranger Station

THRS	N	Mean	SD	SEM	Median
TAOC-before (mmol/L)	20	2.00	0.73	0.16	2.00
TAOC-after	20	1.50	0.36	0.08	1.50
%TAOC difference	20	-21.00	12.22	2.73	-25.00
CMI pos-before (Number)	18	2.89	1.32	0.31	3.00
CMI pos-after	18	3.33	1.28	0.30	3.00
CMI pos-difference (%)	19	41.23	84.87	19.47	0
CMI score-before	17	4.11	0.72	0.17	4.00
CMI score-after	17	3.89	0.40	0.10	3.80
CMI score difference (%)	19	-1.46	22.14	5.08	-5.88
IL2-before (ng/mL)	20	2.58	1.06	0.24	2.16
IL2-after	20	2.93	1.36	0.30	2.55
IL2 difference (%)	20	20.35	55.63	12.44	10.16
IL6 -before (ng/mL)	13	2.01	1.57	0.44	1.22
IL6 - after	14	1.15	1.08	0.29	1.27
IL6 difference (ng/mL)*	19	0.49	1.81	0.42	0
IL2R - before (U/mL)	19	393.29	105.76	24.26	411.80
IL2R - after	20	483.97	205.70	46.00	439.83
IL2R difference (%)	20	10.84	18.67	4.17	4.06
IGF1 - before (ng/mL)	20	245.75	64.62	14.45	233.99
IGF1 - after	20	219.22	71.57	16.00	203.65
IGF1 difference (%)	20	-8.67	25.85	5.78	-7.82
PIVKA - before (ng/mL)	20	2.08	1.16	0.26	1.72
PIVKA - after	20	2.31	1.33	0.30	2.03
PIVKA difference (%)	20	22.82	56.35	12.6	33.61
Ferritin - before (ng/mL)	20	115.15	57.64	12.89	110.00
Ferritin - after	20	92.1	50.18	11.22	86.50
Ferritin difference (%)	20	-18.71	18.39	4.11	-20.09
Fibronectin - before (ug/mL)	20	276.55	74.58	16.68	244.50
Fibronectin - after	20	219.55	59.76	13.36	203.5
Fibronectin difference (%)	20	-17.21	25.48	5.70	-25.04
Hcys - before (umol/L)	16	17.29	5.04	1.26	16.56
Hcys - after (umol/L)	18	19.34	3.88	0.91	20.08
Hcys - difference (%)	19	11.06	45.49	10.44	4.28
Riboflavin - before EGRAC (%)	19	24.00	12.00	3.00	25.00
Riboflavin - after	19	14.00	15.00	3.00	12.00

THRS	N	Mean	SD	SEM	Median
Riboflavin - difference (%)	20	-30.93	86.97	19.45	-41.89
Vitamin B6 - before EASTAC (%)	19	90.00	15.00	4.00	86.00
Vitamin B6 - after	19	98.00	15.00	3.00	101.00
Vitamin B6 difference (%)	19	10.65	14.18	3.25	5.05

C.2. Group at Danum Valley Field Centre

DVFC	N	Mean	SD	SEM	Median
TAOC-before (mmol/L)	11	4.18	0.25	0.08	4.00
%TAOC-after	11	3.09	0.13	0.04	3.00
%TAOC difference	11	-26.01	1.40	0.42	-25.00
CMIpos-before (Number)	9	2.89	0.78	0.26	3.00
CMI pos-after	9	2.78	0.44	0.15	3.00
CMIpos-difference (%)	10	-0.33	29.36	9.29	0.00
CMI score-before	10	4.07	0.72	0.23	4.05
CMI score-after	10	3.82	0.79	0.25	3.90
CMI score difference (%)	10	-5.07	18.35	5.80	-7.17
IL2-before (ng/mL)	11	2.55	1.07	0.32	2.42
IL2-after	11	2.88	1.10	0.33	2.43
IL2 difference (%)	11	29.67	58.79	17.72	66.98
IL6 -before (ng/mL)	10	2.12	3.03	0.96	1.49
IL6 - after	10	2.09	2.12	0.67	1.10
IL6 difference (ng/mL)*	11	-0.02	2.62	0.79	0.00
IL2R - before (U/mL)	11	421.84	92.47	27.88	405.01
IL2R - after	11	415.87	151.89	45.80	367.7
IL2R difference (%)	11	0.50	8.55	2.58	-1.97
IGF1 - before (ng/mL)	8	215.03	19.78	6.99	220.53
IGF1 - after	8	176.81	18.10	6.40	178.04
IGF1 difference (%)	10	-17.10	12.79	4.05	-17.54
PIVKA - before (ng/mL)	8	2.54	1.08	0.38	2.23
PIVKA - after	8	1.76	0.67	0.24	1.77
PIVKA difference (%)	10	-35.80	38.16	12.07	-45.87
Ferritin - before (ng/mL)	11	73.36	21.02	6.34	69.00
Ferritin - after	11	60.00	26.84	8.09	62.00
DVFC	N	Mean	SD	SEM	Median
Ferritin difference (%)	11	-21.07	22.34	6.74	-17.11
Fibronectin - before (ug/mL)	10	295.10	94.90	30.01	284.5
Fibronectin - after	10	217.1	85.81	27.14	212.00
Fibronectin difference (%)	10	-23.98	29.59	9.36	-25.39
Hcys - before (umol/L)	11	18.91	4.98	1.50	17.20
Hcys - after	11	18.67	7.14	2.15	17.62

DVFC	N	Mean	SD	SEM	Median
Hcys - difference (%)	11	1.15	31.04	9.36	10.67
Riboflavin - before EGRAC (%)	8	33.00	6.00	2.00	31.00
Riboflavin - after EGRAC (%)	8	17.00	8.00	3.00	16.00
Riboflavin - difference (%)	11	-51.22	49.66	14.97	-59.51
Vitamin B6 - before EASTAC (%)	7	89.00	3.00	1.00	88.00
Vitamin B6 - after EASTAC (%)	7	91.00	12.00	4.00	93.00
Vitamin B6 difference (%)	10	7.28	11.18	3.53	5.75

